PROCEEDINGS

OF THE

SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE

VOL. 32. OCTOBER, 1934. No. 1.

Illinois Section.

University of Chicago, June 5, 1934.

7531 P

Injections of Sodium Fluoride on Enamel and Dentin of the Incisor of the Rat.*

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The microscopic anatomy of the incisors was studied in 3 groups of rats:

A. 12 rats that were given 2 to 8 injections of .3 cc. of 2.5% sodium fluoride 24 or 48 hours apart. Age: 90-270 days.

B. 20 rats that were given single injections of .3 cc. of 2.5% sodium fluoride and allowed to live 1 to 48 hours after the administration. Age: 90-270 days.

C. 16 controls. Most of these were littermate controls.

Significant alterations were observed only in Groups A and B. The histologic changes in group A were:

1. Both the enamel and dentin show a pair of light (disturbed) and dark (recovery) incremental layers for each injection of so-

* Technical bulletin No. 52 of the University of Arizona Agricultural Station gives a more complete description of some of the phases of this report.

dium fluoride. The width of each pair is approximately 32μ for injections given 48 hours apart and approximately 16μ for injections given 24 hours apart.

2. The light layers represent the immediate response to the injections and are imperfect in formation and calcification.

3. The dark layers represent a recovery response and are normal in formation and normal or excessive in calcification.

4. This incremental pattern is a constant finding but shows disturbances when the administration is continued for more than 5 injections at 24-hour intervals.

The histologic changes in group B varied with the time interval between the injection and death:

1. 48-hour interval. One pair of light and dark incremental layers in the enamel and dentin.

2. 12 to 24-hour interval: a. the incremental surface of the organic enamel matrix lacks its normal arrangement and is covered with hemispherical globules that stain deeply with hematoxylin; b. an abnormal character and distribution of globules within the ganoblastic layer situated in the posterior portion of the incisor.

3. 1-6-hour intervals. Abnormal character and distribution of globules within the ganoblastic layer of the posterior and formative portion of the incisor.

The injection of fluorine offers an accurate and easy method of measuring the rate of growth of the enamel and dentin in continuously growing teeth.

It is believed that fluorine exerts a direct local action on the enamel-forming cells and that the changes observed in the enamel and dentin are not produced primarily by changes in blood calcium and phosphorus. The nature of the cytologic disturbances is being investigated further.

7532 P

Bleeding Volume in Experimental Burns.

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From the Douglas Smith Foundation and the Department of Surgery, University of Chicago.

Roome, Keith, and Phemister[1] showed that in experimental shock due to hyperventilation, anaphylaxis, histamine administration, spinal cord section, and spinal anesthesia the bleeding volume averaged 49.9% of the calculated blood volume (one-thirteenth of the body weight). This is only slightly less than the average of 58.6% obtained for control dogs. On the other hand, in shock due to trauma to an extremity, hemorrhage, plasmapheresis, and intestinal manipulation, the bleeding volume was greatly reduced, averaging 21.8%. Blalock[2] showed that the cardiac output in shock due to severe burns is markedly reduced. The work reported in the present paper was done to determine the bleeding volume in shock due to experimental burns.

Dogs under complete barbital anesthesia and suffering no pain were used throughout the work. They were burned and at intervals the carotid blood pressure, hematocrit reading, and hemoglobin percentage were determined. When the blood pressure had fallen to about 80 mm. of mercury the animal was bled to death through a large carotid cannula. The time interval between the burning and the bleeding varied from 16 to 24 hours in 7 dogs. The hematocrit reading and hemoglobin percentage rose steadily from the time of burning to the time of bleeding. On the other hand, the blood

TABLE I.
Bleeding Volumes of Dogs with Experimental Burns

No.	Wt., Kg.	Interval from burning to bleeding	Blood pressure mm. Hg.		Terminal bleeding vol.
			Start	End	% cal. blood vol.
		hr. min.			
1	10.4	17 40	100	52	11.9
2	9.2	16 15	148	44	14.6
3	11.3	17 50	154	108	29.3
4	7.8	23 10	116	48	16.1
5	10.5	21 40	162	66	16.7
6	12.1	23 45	148	86	28.4
7	6.8	18 20	118	66	24.9
Aver.					20.3

[1] Roome, N. W., Keith, W. S., and Phemister, D. B., *Surg. Gynec. and Obstet.*, 1933, **56**, 161.

[2] Blalock, A. PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 36.

pressure usually remained near the normal level until the end of the experiment and then fell more rapidly. The average bleeding volume in the 7 burned dogs was 20.3% of the calculated blood volume (one-thirteenth of the body weight) as shown in Table I. This figure agrees quite well with that of 21.8% which Roome, Keith, and Phemister found in similar types of shock. The extent of the burn in these dogs may be gauged as follows: After death the dogs were bisected according to the method of Blalock[3] and the weight of the normal and burned sides of the animal compared. This difference was 3.0, 2.5, 2.2, 0.3, 1.1, 3.0, and 2.4, averaging 2.1% of the total body weight in the 7 dogs and represents a fluid loss into the tissues on the burned side sufficient to account for the shock present in these animals.

In 4 control dogs that were similarly treated except that they were not burned, the average bleeding volume was 53.4% of the calculated blood volume as shown in Table II. This figure agrees

TABLE II.
Bleeding Volumes of Control Dogs.

No.	Wt. Kg.	Interval from start exper. to bleeding	Blood pressure mm. Hg. Start	Blood pressure mm. Hg. End	Terminal bleeding vol. % cal. blood vol.
		hr. min.			
1	24.0	24 0	154	128	49.8
2	13.0	17 20	134	136	52.0
3	12.1	15 40	136	102	47.2
4	19.5	18 20	118	116	64.7
Aver.					53.4

rather well with that of 58.6% which Roome, Keith, and Phemister found for control dogs. Any slight discrepancy may be explained by the fact that their dogs were bled one hour after beginning the experiment, whereas in the present paper the time interval in the control series was from 16 to 24 hours.

The blood pressure, hematocrit reading and hemoglobin percentage fell very slightly during this interval.

From a comparison of the results in the burned and control series it is concluded that the bleeding volume is markedly reduced in experimental burns.

[3] Blalock, A. *Arch. Surg.*, 1931, **22**, 610.

7533 P

Toxicity of Heavy Water.*

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The following experiments were performed in an effort to determine whether or not the newly recognized substance, heavy water (deuterium oxide) possesses toxic properties. Water containing approximately 20% deuterium oxide was prepared electrolytically and its action tested on rabbit leucocytes and bacteria. Experiments are now being done testing heavy water in more concentrated form up to 100%.

Experiment 1—Rabbit leucocytes. One drop of a 24-hour aleurinate broth exudate, freshly removed from the pleural cavity of a rabbit, was added to 2 cc. of a modified Tyrode solution containing 18% heavy water and 1/10,000 neutral red. The Tyrode solution, which was buffered at pH 6.6, had 10% of its salts replaced by NH_4Cl. Two hanging drops and one spread preparation, sealed with vaseline-paraffin mixture, were incubated at 37°C. and observed under the microscope. A control series was prepared with triple distilled water.

The viability of the leucocytes was followed. After one hour an occasional dead cell could be seen. In 8 hours 10% were dead; in 16 hours 50%, and in 24 hours about 75% were dead. There were no significant differences between leucocytes in the heavy water solution and control ordinary water preparations.

Experiment 2—Growth of Staphylococcus. A. A minute amount of an 18-hour broth culture of hemolytic *Staphylococcus aureus* was added to a drop of 20% heavy water. The drop was divided into 3 parts which were sealed on sterile cover slip hanging drops. These were cultured on blood agar plates after 6, 24 and 48 hours respectively. There was no inhibition of growth.

B. A drop of 20% heavy water was placed in the center of a blood agar plate freshly streaked with the organism used in A. No effect was noted during a period of 4 days.

Experiment 3—Motility of B. Typhosus. A shaked loop of an 18-hour ascitic-broth culture of *B. typhosus* was stirred into 2 hang-

* Work done in part under a grant from the Douglas Smith Foundation.

ing drop preparations of heavy water and into 2 controls with distilled water. Over a period of 48 hours no difference in motility could be observed between the heavy water and control ordinary water preparation.

7534 P

Depressor Substances in Peritonitis.

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There is little experimental work to substantiate the clinical belief that death from peritonitis is due to vasomotor collapse incident to absorption of toxins from the peritoneum. Zinnser, Parker and Kuttner[1] and Branham[2] both demonstrated that *Escherichia coli* produced a soluble toxic substance. Steinberg and Ecker[3] and Steinberg[4] have emphasized the rôle of bacterial toxins in peritonitis. Steinberg and his co-workers[5] have demonstrated a slight blood pressure fall in early peritonitis. Scott and Wangensteen[6] have showed that the peritoneal exudates from uncomplicated experimental intestinal obstruction were innocuous.

It occurred to us that the vasomotor system of the host might be less sensitive than that of a normal animal to the toxic substances developed in the peritoneal cavity. Peritonitis was induced in 17 dogs by the method of Buchbinder, Heilman and Foster[7] which consists of leaving an open loop of ileum with intact blood supply free in the peritoneal cavity. An end to end or a lateral anastomosis is made around the loop to restore the continuity of the intestinal

* Supported in part by a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Zinnser, H., Parker, J. T., and Kuttner, A., PROC. SOC. EXP. BIOL. AND MED., 1920, **18**, 49.

[2] Branham, S. E., *J. Infect. Dis.*, 1925, **37**, 538.

[3] Steinberg, B., and Ecker, E. E., *J. Exp. Med.*, 1926, **43**, 443.

[4] Steinberg, B., *Arch. Surg.*, 1931, **23**, 145.

[5] Steinberg, B., Kobacker, J. L., and Russel, T. G., PROC. SOC. EXP. BIOL. AND MED., 1930, **30**, 1155.

[6] Scott, H. G., and Wangensteen, O. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 559.

[7] Buchbinder, J. R., Heilman, F. R., and Foster, G. C., *Surg. Gyn. and Obst.*, 1931, **53**, 726.

tract. Bile peritonitis was induced in 9 dogs by 3 methods: by the intraperitoneal injection of whole sterile dogs' bile, by the similar administration of a sterile bile salt solution and by ligature of the common bile duct followed by defundation of the gall bladder. Twenty-three specimens of peritoneal washings or of peritoneal exudates were obtained by aseptic lavage of animals with suppurative peritonitis some time prior to death. These fluids were then centrifuged at high speed until clear and the supernatant fluid injected intravenously into normal dogs under barbital or urethane anesthesia. In a few instances the supernatant fluid was passed through a Mandler filter of porosity corresponding to a Berkefeld-N filter. Since the effect on blood pressure of the Mandler filtrates differed only quantitatively from the centrifuged supernatant fluid, the 2 will be considered collectively. As a control 18 fluids were obtained by aseptic lavage of the peritoneal cavity of 13 normal dogs. Extracts were made by the method of Chang and Gaddum[8] of the whole peritoneal washings, the supernatant fluids, and of the centrifuged sediment from all the above materials.

The symptoms described by the investigators[1-3] who demonstrated the presence of the soluble toxic substance of *Escherichia coli* are compatible with those produced by a profound drop in blood pressure. Accordingly, we have also investigated the hemodynamic effects of bacterial filtrates of broth cultures of *Escherichia coli* and of a hemolytic streptococcus freshly isolated from a case of streptococcus peritonitis in man. Extracts by the method of Chang and Gaddum[8] were also prepared of the corresponding bacterial sediments in several instances. Peptone-free veal infusion broth was used for the nutrient medium. Aerobic and anaerobic cultures were made in every instance when the peritoneal cavity was opened. In those cases where a significant drop in blood pressure was obtained upon injection of the peritoneal washings into a normal dog there was a heavy growth of *Escherichia coli* with or without a growth of an obligate anerobe resembling *Clostridium welchii.*

Results. *(a) Peritonitis induced by an open intestinal loop.* In 15 instances taken from tests of the 23 specimens of peritoneal washings from dogs with peritonitis produced by an open intestinal loop, there was a profound but variable immediate drop in blood pressure of from 18 to 80 mm. of mercury. Three specimens obtained post-mortem varied from the others in that the latter samples were tainted by the odor of putrefaction. These latter 3 gave an especially rapid and profound drop in blood pressure. A considera-

[8] Chang, H. C., and Gaddum, J. H., *J. Physiol.*, 1933, **79**, 255.

tion of the first appearance of vasodepressing substance indicates that an appreciable time is required to develop vasodepressing substances in the peritoneal content. The control washings from normal dogs and from those in which peritonitis was not present had no effect upon the blood pressure of another dog.

(b) Bile Peritonitis. The centrifuged exudate or the combined exudate and washings removed from 8 dogs in which bile peritonitis was present had no depressing effect on the blood pressure of other dogs, while in a single instance vasodepression was noted with a fluid obtained from an animal that had died a few hours prior to lavage of the peritoneal cavity.

(c) Extracts of Peritoneal Exudates, Washings and Sediments. Extracts of these materials prepared according to the method of Chang and Gaddum[8] demonstrated the presence and concentration of a vasodepressing substance in the whole exudate and in the centrifuged sediments at all times, both from the bile peritonitis animals and those with suppurative peritonitis. A vasodepressant extract was obtained from the supernatant centrifuged fluid only when that fluid itself contained such substances. Bacterial sediments contained no vasodepressing substance. The finding of such a substance in normal mammalian tissues is in agreement with the finding of such substances by Harkins and Harmon.[9]

(d) Bacterial Filtrates. Without exception a vasodepressing substance with a delayed time of action of 20 to 45 minutes after injection was present in Mandler filtrates of *Escherichia coli.*

7535 P

Mutual Influence of Crystalloids of Human Blood Serum on Their Equilibrium.

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The blood serum is an aqueous (ultramicroscopic)-suspension of colloids in a solution of crystalloids. The crystalloids are under normal conditions in a well balanced equilibrium, which is sustained by the colloids: static equilibrium. A normal blood serum for

[9] Harkins, H. N., and Harmon, P. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 6.

instance contains 90-120 mg. of dextrose per 100 cc. of blood. The presence of the colloids inhibits even *in vitro* an increase of this amount within certain (physiological) limits. If we try to increase the blood sugar by adding the physiological amount of 100 mg. per 100 cc. and determine the sugar we find only 25% of the added amount; 75% (60-80%) disappear in the normal human serum. The serum colloids have, therefore, the power to restore and to maintain the equilibrium, if we try to disturb it: dynamic equilibrium. The following studies are based on this observation. The serum crystalloids are electrolytes and non-electrolytes. The influence of changes of the electrolytes as well as of the non-electrolytes on the static and on the dynamic equilibrium of the non-electrolytes of the normal human blood serum has been studied with the following results:

(1) Influence of cations on the static equilibrium of the serum sugar: Chlorides have been used in n/10, n/100 solution, 0.02 cc. added to 1 cc. of serum. NaCl does not influence the blood sugar even in n-solutions. KCl increases the blood sugar (5-10%), $CaCl_2$ and $MgCl_2$ do not influence the sugar as a rule, but act differently in different sera. $FeCl_3$ always decreases the sugar of a normal serum, ca. 10%.

(2) The influence of cations on the dynamic equilibrium of the serum sugar, *i. e.*, after the addition of a physiological quantity (200 mg. per 100 cc.) acts exactly in the same way, but more intensely; NaCl does not influence the loss of the added dextrose, KCl decreases the loss considerably, $CaCl_2$ and $MgCl_2$ also decrease the loss, but less than KCl; $FeCl_3$ always increases the loss of dextrose.

(3) Influence of anions on the static equilibrium of the serum sugar: The neutral sodium salts of the anions have been added in the same amounts as used in the cations (n/10 solutions, 0.02 cc. to 1 cc. of serum). Chloride and carbonate do not influence the serum sugar, phosphate and sulphate liberate sugar in a similar way as KCl; Na_2SO_4 has a stronger action than Na_3PO_4.

(4) Influence of anions on the dynamic equilibrium of the serum sugar: The only salt which had a marked influence was Na_2SO_4, which decreased the loss of dextrose considerably.

(5) Influence of cations on the static equilibrium of the urea of the serum: $FeCl_3$ has a pronounced decreasing influence upon the urea of the serum, $CaCl_2$ has no influence, $MgCl_2$ and also KCl do not influence, as a rule, the original urea of the serum, there are, however, considerable differences among the different sera.

(6) Influence of cations on the dynamic equilibrium of urea:

Urea added to the serum can be quantitatively recovered; there is no loss like in dextrose. $FeCl_3$ causes a loss of the urea added, the other cations do not influence the dynamic equilibrium.

(7) Influence of anions on the static equilibrium of urea: Na_2CO_3 causes a loss of ca. 10%, Na_2SO_4 and Na_3PO_4 cause a loss up to 50% of the original urea of the serum.

(8) Influence of the anions on the dynamic equilibrium of the urea: The 3 sodium salts examined all had the same influence on the urea, causing the loss of about 7% of the urea added.

(9) There is an interesting antagonism between the serum dextrose and the serum urea, concerning their influences on cations and anions: Adding of dextrose increases the influence of cations and nullifies the influence of anions on urea. Adding urea decreases the influence of cations on dextrose, nullifies the influence of Na_2SO_4, but intensifies the influence of Na_2CO_3 and of Na_3PO_4 on dextrose.

(10) Influence of non-electrolytes on non-electrolytes (mutual influence of dextrose and urea): Adding dextrose to the serum causes a loss of dextrose, as mentioned, as high as 75%. It does not, however, influence the serum urea (static equilibrium). There is no loss of urea, when urea is added (30 mg. per 100 cc.), and none of dextrose. The simultaneous addition of urea and dextrose does not alter the loss of dextrose which remains 75%, but causes a loss of the added urea (dynamic equilibrium) between 30 and 50% in a normal human serum.

All these experiments have been checked in aqueous solutions. The presence of the serum colloid is essential for the results. According to previous studies of the author[1] it is very likely that the struggle for water between the highly hydrophilic and even hygroscopic electrolytes and non-electrolytes plays an important rôle in the maintenance of their equilibrium in the presence of serum. The quantities added were exceedingly small: 0.15 mg. of KCl, 0.32 mg. of $FeCl_3$ have been added, but even higher dilutions—0.03 mg. of $FeCl_3$ proved to be effective. The importance of these studies for the "Micrometabolism" (Wright[2]) in different diseases will be reported elsewhere.[4, 5] Anabolic and catabolic influences of drugs can easily be determined with these methods, *in vitro* as well as *in vivo*.

[1] Pribram, E., *Z. f. Kolloidchemie, Beih.*, 1911, **2**, 1.

[2] Wright, F., *Clin. Med. and Surg.*, 1933, **40**, 517.

[4] Pribram, *Arch. f. Gewerbepathologie*, 1934, **5**, 23.

[5] Pribram, *Schweizer. Wochenschr.*, 1934, in print.

7536 P

Urea, Promoter of the Catalytic Action of Blood Serum on a Specific Dextrose-Phosphate Reaction.

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Dextrose and phosphates have a high tendency to react. They are both present in animal and human tissue as well as in the blood serum. When mixed in an aqueous solution in the same quantities, which are present in the blood serum, *i. e.,* 3 mg. of P in the form of a neutral phosphate and ca. 100 mg. of dextrose per 100 cc., we find a very slight reaction, which can be assumed because we recover a little less of dextrose and a little less of phosphorus than we do when the chemicals are added separately; 2.8 mg. of P are recovered instead of 3 mg. per 100 cc., and 90 mg. of sugar instead of 100 mg. The same quantities added to human serum of a healthy individual react much more intensely. Instead of the original quantities added we recover only 25-50% of dextrose and ca. 70% of phosphorus. The loss of dextrose has been explained in the previous paper. It is caused by the increase of dextrose, which is counteracted by the serum colloids. The loss of phosphorus, however, does not occur if no sugar is added. In this case we recover 100% of the added phosphate. Urea added to the serum does not influence the serum phosphate, and also does not influence the dynamic equilibrium of the phosphate, when this salt (3 mg. per 100 cc.) and at the same time urea (30 mg. per 100 cc.) are added. We still recover the entire phosphate. The reaction between dextrose and phosphate, however, is intensely accelerated and intensified, if phosphate, urea and dextrose are added to the serum. In this case only a small quantity of the phosphate, 10%, and frequently none of the added salt can be recovered, provided the serum is that of a normal healthy individual. The loss of the dextrose is not influenced by the urea, but there is a loss of the urea as high as 30-50%, which, as stated previously is caused by the adding of dextrose.

Pathological conditions, in which the physiological reaction of dextrose with phosphate remains incomplete will be considered in another article.

The catalytic action of the serum colloids and their promotion by the urea are specific for dextrose. Other saccharides, such as galactose or lactose, do not react with the phosphate, but allow 100% of

the added phosphorus to be recovered, even when urea is added besides the sugar. It is evident that we deal here with an important anabolic influence of the urea, which, in the serum or tissues, is not merely a waste product, as it is as a rule considered in the urine.

7537

Behavior of Blood Cholesterol Following Injections of Tuberculin.

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The literature gives but scant attention to the relation of cholesterol to tuberculosis. It has been found by a few observers that the blood cholesterol is definitely decreased during the activity of the tuberculous process, and this hypocholesterolemia, therefore, may be regarded as an index of poor prognosis. With the improvement of the pathologic process, however, the blood cholesterol rises and during convalescence it may be increased above the normal level. The present experiments endeavor to study the behavior of total blood cholesterol in rabbits following injections of old tuberculin.

A total of 10 rabbits were divided into 2 groups. The first group of 6 rabbits (average weight 1660 gm.) received single injections of 1:10 dilution of old tuberculin, and the blood cholesterol was determined daily by the Bloor method as modified by Sackett. The results are summarized in Table I.

The second group of 4 rabbits (average weight 3340 gm.) were first sensitized by repeated injections of old tuberculin over periods varying from 10-60 days. After rest periods of 2-6 months, these animals were given single injections of tuberculin and the blood

TABLE I.
Blood Cholesterol in Unsensitized Animals.

Observed	Female 1725 gm.	Female 1400 gm.	Male 1750 gm.	Female 1725 gm.	Female 1725 gm.	Female 1650 gm.
5-26	138	113	100	118	120	150
5-28	202	212	225	—	275	200
5-29	192	300	137	202	170	267
5-29	0.2 cc. 1/10 O.T.		1.0 cc. 1/10 O.T.		3.0 cc. 1/10 O.T.	
	Intraven.	Subcut.	Intraven.	Subcut.	Subcut.	Intraven.
5-30	313	380	300	332	375	288
5-31	201	282	283	289	263	220
6-1	190	267	287	240	197	250
6-2	195	325 (?)	195	193	203	215
6-3	193	260	267	214	195	312

cholesterol was followed by the same method as in the first group of animals. The results are summarized in Table II.

TABLE II
Blood Cholesterol in Sensitized Animals

Observed	Female 3400 gm.	Female 3150 gm.	Male 3250 gm.	Female 3550 gm.
5-15	174	273	170	207
5-16	155	269	197	173
5-17	149	275	181	—
5-17	0.1 cc. 1/10 O.T. Intraven.	0.5 cc. 1/10 O.T. Subcut.	1.0 cc. 1/10 O.T. Intraven.	1.0 cc. 1/10 O.T. Subcut.
5-18	255	330	375	156
5-19	325	415	675	415
5-21	373	547	266	357
5-22	200	—	145	110
5-23	231	347	162	150
5-24	195	168	110	100
5-25	148	165	107	83
5-29	215	225	150	165
6-1	250	235	153	230
6-3	275	287	265	150

The results indicate that these animals respond to the single injections of tuberculin with prompt though transient hypercholesterolemia. This is followed within a week by a return to normal value, which in some instances is first preceded by a fall below the normal level.

7538 C

Bactericidal Power of Blood in Chronic Arthritis.

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There is considerable evidence that chronic rheumatic joint disease, as well as acute rheumatic arthritis, is neither strictly a metabolic disturbance nor purely allergic in character but the result of hematogenous streptococcic infection of the joints. This evidence is of 4 types: the demonstration of streptococci in involved joints with typical structural alteration in direct relation to the actual distribution of the bacteria;[1, 2, 3] the streptococcemia which occurs at

[1] Forkner, C. E., Shands, H. R., and Poston, Mary A., *Arch. Int. Med.*, 1928, **42**, 675.

[2] Cecil, R. L., Nicholls, E. E., and Stainsby, W. S., *Arch. Int. Med.*, 1929, **43**, 571.

[3] Wetherby, M., and Clawson, B. J., *Am. J. Path.*, 1932, **8**, 283.

intervals during the active phases of the disease;[2, 4, 5] the presence of streptococcic antibodies in arthritic patients in concentrations greater than those usually found in normal individuals;[5, 6, 7, 8] and the production experimentally of lesions in the joints of animals by the use of intravenous injections of streptococcus cultures.[2, 10] On the other hand, other investigators have failed to corroborate some of these observations.[11–13]

This positive evidence has led to the wide use of agglutination and sedimentation tests as diagnostic procedures in chronic arthritis and to the use of vaccines for treatment of the disease. Our own experience and that of others[7] with the use of agglutination tests and determinations of sedimentation rates have led us to believe that these have little practical value in diagnosis or as indices of the results of treatment. In searching for a more reliable index of immunity to streptococcic infection, we have tested the whole or defibrinated blood of 30 patients and the serum of 38 patients from the Evanston Hospital Arthritis Clinic for bactericidal power. As controls, 31 tests on the defibrinated blood and 22 on the serum of normal individuals were done. Bactericidal tests were performed also on the blood and serum of 10 patients suffering from non-streptococcic infections and in patients with acute streptococcic sepsis. In most instances the sedimentation rate and the agglutinating titer were determined on samples of blood drawn at the same time for comparison with the results obtained in the bactericidal tests.

Preliminary experiments showed that there was little difference between the bactericidal power of heparinized blood and that of defibrinated blood if the defibrination was done carefully and with constant technique. The latter method was finally adopted for routine tests because the procedure was simple and inexpensive.

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[4] Richards, J. H., *J. Am. Med. Assn.*, 1925, **84**, 637.

[5] Gray, J. W., Fendrick, E., and Gowen, C. H., *Texas State Med. Journal*, 1932, **28**, 317.

[6] Burbank, R., and Hadjopoulos, L. G., *J. Am. Med. Assn.*, 1925, **84**, 637.

[7] Dawson, M. H., Olmstead, Miriam, and Boots, R. H., *J. Immunol.*, 1932, **23**, 187.

[8] Clawson, B. J., Wetherby, M., Hilbert, E. H., and Hilleboe, H. E., *Am. J. Med. Sc.*, 1932, **184**, 758.

[9] Keefer, C. S., Meyers, W. K., and Oppel, T. W., *J. Clin. Invest.*, 1933, **12**, 267.

[10] Burbank, R., *Bull. N. Y. Acad. Med.*, 1929, **5**, 176.

[11] Nye, R. N., and Waxelbaum, E. A., *J. Exp. Med.*, 1930, **52**, 885.

[12] Dawson, M. H., Olmstead, Miriam, and Boots, R. H., *Arch. Int. Med.*, 1932, **119**, 173.

[13] Bernhardt, H., and Hench, P. S., *J. Infect. Dis.*, 1931, **49**, 489.

An eleven-year-old strain of *Streptococcus viridans* obtained from Dr. B. J. Clawson was used as the test organism chiefly because it had been used previously in our routine agglutination tests. Several of the strains that we have isolated from the blood of arthritic patients have been similar culturally and serologically to this one. Tests with recently isolated strains of streptococcus gave unreliable results by our method.

For the test 1 cc. of a suspension containing about 10,000 bacteria per cubic centimeter was used. This was added to 3 cc. of a 50% dilution of serum, or to 3 cc. of defibrinated blood, in a 7 cc. Wassermann tube. The tube was closed with a paraffined rubber stopper and placed in the rack of a mixing machine in a 37° incubator. Constant agitation was found to be necessary for these tests and a rotating machine was devised for this purpose.*

The degree of reduction of living organisms was determined by the plating method. For this a 0.5 cc. sample of the mixture was removed, plated with 10 cc. of dextrose agar immediately after the mixture was made and again after 4 hours and 8 hours of incubation in the agitating machine. The colony counts were made after 18 to 24 hours of incubation and recorded numerically when possible, or estimated roughly as 2 plus (4000±), 3 plus (6000±) and 4 plus (8000±) when too numerous for actual counting.

The results of the initial tests in arthritic and non-arthritic patients are summarized in Table I. For the sake of simplicity we have merely indicated as having bactericidal power those cases which showed inhibition after 4 hours of rotatory agitation in the incubator. The results varied from complete inhibition to slight (10%) reduction in the number of colonies.

In 38 arthritic patients the serum alone of only 2 cases (5%) exhibited bactericidal power, while in 3 cases (9%) among the controls definite bactericidal action was observed.

It is interesting that in the group of cases of active arthritis, all of which were of a severe type, only one blood failed to show definite inhibition of the strain used. The agglutination titer in these cases was well within normal limits and the sedimentation rates showed about the same average values and varied within narrower limits than in the normal cases.

In the group of 12 cases of arthritis that showed unequivocal clinical evidence of improvement, only 5 gave positive bactericidal tests. The sedimentation rates varied within the same limits as in the normals. Agglutination titers were not done at this time.

* Details of the technique and a description of the agitating machine are included in an accompanying paper.

TABLE I.
Bactericidal Power, Agglutination Titer and Sedimentation Rate in Arthritis and Non-arthritic Conditions.

			Agglutination Tier				Sedimentation Rate in mm. (60 min.)			
	Total cases	Cases with pos. Bc tests	Av. in pos. cases	Extremes in pos. cases	Av. in neg. cases	Extremes in neg. cases	Av. in pos. cases	Extremes in pos. cases	Av. in neg. cases	Extremes in neg. cases
Normal individuals	31	21 (68%)	96	20-320	44	20-180	11	2-27	15	5-24
Arthritis (untreated)										
Atrophic and mixed — Recovering	12	5 (41%)					17	9-26	14	6-24
Atrophic and mixed — Active	12	11 (91%)	50	20-80			11	2-23		20
Hypertrophic	5	3 (60%)					17	14-19	11	5-21
Arthritis (treated-vaccine)										
Atrophic and mixed — Recovering	12	7 (58%)	1730	320-5120	1664	160-5120	18	4-29	12	6-19
Atrophic and mixed — Active	2	2 (100%)	1600	640-2560			4	3-5		
Hypertrophic	6	3 (50%)	320	160-640	3630	640-5120	16	14-19	12	5-21
Streptococcic sepsis	2	2 (100%)	160	0-320						
Non-strep. infections										
Acute	6	5 (83%)	104	0-320		80	14	4-28		26
Chronic	4	3 (75%)	50	20-80			10	3-18		6

Of the 5 cases diagnosed as hypertrophic arthritis 3 gave positive bactericidal tests and the sedimentation rates were not significant.

The acute and chronic non-streptococcic infections were local lesions, some with suppuration but none showing systemic effects. In 3 of the chronic cases the infection was localized. In these cases agglutination tests yielded normal values and the sedimentation rate, while slightly greater in the acute infections, varied within narrow limits. The bactericidal effect of the defibrinated blood in the more acute cases is comparable with that in the active cases of infectious arthritis. This evidence supports the idea that the bactericidal property of whole blood or defibrinated blood as applied to gram positive cocci is not specific.

Both of the cases of acute streptococcic sepsis exhibited marked bactericidal power. In both instances *Streptococcus viridans* was recovered repeatedly in blood cultures. It is interesting that in the patients with acute streptococcic or non-streptococcic infections and in the cases of active arthritis, the bactericidal power of the blood was approximately the same. The agglutination titers in these 2 patients with streptococcus sepsis varied within normal limits, emphasizing again the lack of correlation between bactericidal power and the agglutination titer.

In the third division of the table the data accumulated from studies of 20 arthritic cases receiving intravenous vaccine therapy at the time of the initial bactericidal tests, are summarized. As in past experience we find generally increased agglutination titers without correlation between titer values and clinical improvement. The 2 most active cases showed the lowest sedimentation rates. Vaccine therapy apparently had no effect upon either bactericidal power or sedimentation rate.

Conclusions from such small groups of cases as presented in the table must necessarily be guarded. However, we consider that the following generalizations are justified in the light of previous findings of other workers and supplementary unpublished data from our own experience.

1. Tests of bactericidal power of blood by the method used are of little or no value in the diagnosis of chronic arthritis.

2. In general, the defibrinated blood from active cases of atrophic arthritis and acute streptococcic and non-streptococcic infections more often exhibits inhibitory properties against *Streptococcus viridans* than the blood from normal individuals, from patients with

chronic non-streptococcic infections, from quiescent or recovering cases of atrophic arthritis, or from hypertrophic arthritis.

3. Intravenous streptococcic vaccine therapy does not influence the bactericidal property of blood while the agglutinating titer may be greatly increased.

4. There is no correlation between streptococcic bactericidal property, agglutinating titer and sedimentation rate in the cases so far examined.

5. Serum alone usually shows little or no bactericidal power against *Streptococcus viridans,* irrespective of its agglutinating titer. In exceptional cases there may be definite inhibitory action.

7539 C

Importance of Continuous Agitation in Bactericidal Tests with Streptococci.

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Although the advantages of continuous agitation in phagocytosis experiments had been noted by earlier investigators,[1, 2, 3] Robertson and Sia[4] were apparently the first to use the rotatory-oscillation method extensively. All have observed that constant mixing during incubation promotes bactericidal action and produces more constant results than stationary incubation or intermittent agitation. Most of the previous work has been done with cultures of pneumococci and we were faced with the necessity of checking these factors with the streptococcus with which we were working.

An agitating machine utilizing the same principle as that in the machine used by Fenn[2] and later in the improved machine of Robertson and Sia[4] was designed and built for us by Mr. Wm. H. Hamilton, E. E. It consists of 3 brass flanges mounted rigidly on a motor driven shaft. Two of the flanges act as tube holders, having 5/8 in. perforations bored at uniform intervals near the periphery, while the third flange acts as a guard. The flanges will hold 18 tubes at

[1] Rosenow, E. C., *J. Infect. Dis.*, 1906, **3**, 683.

[2] Fenn, W. O., *J. Gen. Physiol.*, 1920, **3**, 439.

[3] Kite, G. L., and Wherry, W. B., *J. Infect. Dis.*, 1915, **16**, 109.

[4] Robertson, O. H., and Sia, R. H. P., *J. Exp. Med.*, 1924, **39**, 219.

one time and the angle of the tubes can be altered by placing one end of the tube in the second or third perforation from that opposite the one containing the other end of the tube. The tubes are held in place by a rubber band which encircles them. A constant speed electric fan motor furnishes the power which is transmitted by pulley through a short belt (rubber band) and a worm and circular gear.

The rotating rack revolves at a constant speed of 18 r.p.m. and the mixtures shift from the base of the tube to the top against a paraffined rubber stopper and back again during each revolution. The chief advantages of this design are the mechanical simplicity, constant speed and small space occupied by the machine. It fits conveniently on the top shelf of a small incubator and requires no more attention than occasional oiling of the motor (Fig. 1).

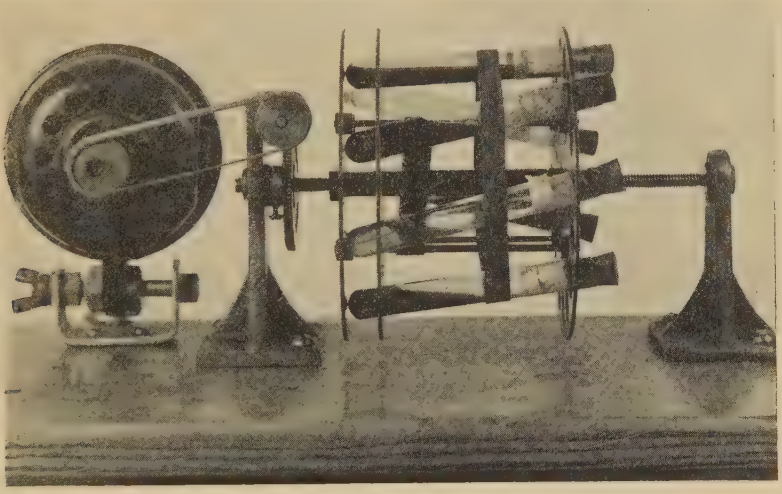

FIG. 1.

Tests were performed with defibrinated blood and washed suspensions of 24-hour cultures of *Streptococcus viridans,* with and without agitation in the machine. The mixtures were made in the proportion of 3 cc. of freshly drawn, defibrinated blood and 1 cc. of gelatin-Locke's solution containing about 10,000 bacteria. A 0.5 cc. sample of this mixture was plated with 10 cc. of dextrose agar immediately and again after 4 hours of incubation. The results are indicated in Table I. In the stationary tubes an actual reduction in the number of bacteria as indicated by the colony count was noted in only 2 cases of the 19 tested. In every case a much greater killing effect was obtained in the mixtures that were rotated in the machine.

TABLE I.
Bactericidal Effect With and Without Rotatory Agitation.

Case No.	Colony Increase or Decrease in % Stationary	Rotatory Agitation
1 (F.W.)	+100	—100
2 (K.S.)	+1220	+59
3 (Z.W.)	+290	—78
4 (T.S.)	+1330	—98
5 (W.F.)	+1330	+1140
6 (A.H.)	+1000	+44
7 (D.C.)	+2000	+100
8 (I.H.)	+540	—99
9 (A.F.)	+2000	+690
10 (M.S.)	+2000	+260
11 (M.K.)	—75	—100
12 (W.H.)	+44	—75
13 (R.H.)	+322	—100
14 (M.S.)	+270	—100
15 (M.B.)	+110	—100
16 (G.M.)	+51	—100
17 (C.C.)	+127	+80
18 (A.R.)	—68	—100
19 (A.A.)	+200	+122

Similar differences were noted in 7 tests in which, instead of incubating in stationary tubes after the initial thorough mixing, the tubes were mixed by inverting several times by hand at 15-minute intervals and allowed to settle in racks in the interim (Table II).

TABLE II.
Bactericidal Effect with Intermittent Shaking and Rotatory Agitation.

Case No.	Colony Increase or Decrease in % Intermittent Shaking	Rotatory Agitation
1 (F.L.)	+60	—100
2 (D.P.)	+100	—100
3 (G.E.)	—12	—100
4 (E.M.)	+300	—99
5 (J.E.)	—56	—100
6 (Q.L.)	+2000	+43
7 (M.S.)	+1000	—99

In every instance the colony count was less in mixtures rotated constantly in the agitating machine.

7540 P

Studies on Enzymatic Digestion of Gastric Mucin.

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Although it is generally assumed that mucin is digested in the intestinal tract, no systematic investigations on the enzymatic hydrolysis of mucin have been reported. We have, therefore, undertaken to study the action of enzymes upon mucin *in vitro* for the purpose of determining its possible manner of cleavage.

The general procedure consisted in adding a known amount of enzyme to a mucin solution of known concentration at a pH well within the range of activity of the enzyme being tested. Controls consisted of mucin alone and enzyme alone at approximately the same pH. At intervals, usually 2 days, samples were removed and analyzed, until no further breakdown beyond that occurring in the controls could be detected. Proteolysis was followed by the nitrous acid method of Van Slyke.[1] Cleavage of the carbohydrate portion of the molecule was tested for by determining the presence or absence of reducing substances, using the Somogyi-Shaffer-Hartmann method.[2] The extent of cleavage was also determined by the decrease in the amount of mucin precipitable in 70% alcohol, and subsequent analysis of the precipitated material. The activity of all enzymes used was demonstrated by their hydrolysis of suitable substrates. In those cases in which digestion of mucin was observed, a second portion of enzyme was subsequently added to preclude the possibility that cessation of hydrolysis was due to depletion or inactivation of the enzyme.

We have previously reported the isolation of a purified mucin from commercial preparations by a process of peptic digestion followed by 70% alcohol precipitation.[3] Since peptic digestion is used in the preparation of commercial mucin, the additional peptic digestion was employed for the sole purpose of rendering alcohol soluble any extraneous protein material present as a result of improper manufacture, or subsequently added as a diluent. In the

[1] VanSlyke, D. D., *J. Biol. Chem.*, 1929, **83**, 425.

[2] Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Baltimore, 1932, **2**, 469.

[3] Anderson, R. K., Fogelson, S. J., and Farmer, C. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 518.

absence of these conditions, identical material can be obtained by alcohol precipitation, either with or without additional peptic digestion. The mucin used in these investigations was prepared from an undiluted commercial preparation by precipitation in 70% alcohol. Mucin prepared in this manner was entirely resistant to further peptic action. It is quite possible that commercial mucin represents merely the peptic resistant portion of native mucin, though no conclusive evidence is available on this point.

In studying the action of trypsin upon mucin, enzyme preparations from 3 different commercial sources were employed. One sample was tested and contained, in addition to trypsin, the ereptic enzymes dipeptidase and aminopolypeptidase. The data obtained with all 3 preparations were practically identical, the amounts of amino nitrogen liberated being equal to 7.57%, 7.90%, and 7.85% of the total nitrogen of the mucin.

The commercial erepsin employed was found to contain, in addition to erepsin, a small amount of proteinase. When added to a tryptic digestate of mucin, the additional amino nitrogen liberated was equal to approximately 3% of the total nitrogen, or a total increase of approximately 11% due to the combined action of trypsin and erepsin.

Table I summarizes the results when cleavage was followed by means of 70% alcohol precipitation and subsequent analysis of the precipitated material. These data, in agreement with those obtained by the Van Slyke method, indicate partial digestion by both trypsin and erepsin.

TABLE I.

%Precipitation, Nitrogen and Reduction of Mucin Digestates.

	% Precip.*	% N.	% Reduction† (as glucose)
Before digestion	83.2	7.15	34.5
After tryptic digestion	76.5	6.9	38.2
Tryptic + ereptic digestion	73.4	6.2	44.2

* By alcohol at 70% concentration.

† After acid hydrolysis.

Proteolytic enzymes were also prepared from yeast by water extraction and their action upon mucin tested. An increase in amino nitrogen equal to 15.1% of the total nitrogen was observed.

The following enzymes were tested with negative results: maltase (alpha glucosidase), emulsin (beta glucosidase), steapsin and pancreatic amylase.

An intestinal extract was prepared by water extraction of the duodenum and first few inches of the ileum of a dog. The extent of digestion was practically identical with that caused by combined action of commercial trypsin and erepsin. The addition of these enzymes produced no further cleavage. No digestion of the carbohydrate portion of the molecule was observed.

These studies indicate that mucin, in contrast to most proteins, is relatively resistant to enzymatic hydrolysis *in vitro,* a property in accord with its ascribed protective action. However, it seems probable that further digestion of mucin occurs in the digestive tract. This is indicated by the fact that normally no readily detectable quantities of mucin are excreted from the intestinal tract, and, furthermore, that glucuronic acid of mucin is available for conjugation.[4] Perhaps specific enzymes exist for this purpose, or some definite but at present unknown sequence of enzymatic action is required. These studies are being continued with purified enzyme preparations.

7541 C

Depressor Extracts of Some Human Tissues.*

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From the Department of Surgery, The University of Chicago.

The recent work of Dale, Dudley, and their associates[1, 2, 3] has thrown new light on the depressor substances that may be extracted from animal tissues. The methods of preparation of and differentiation between the various depressor substances have been described especially by Chang and Gaddum.[1] Because these methods are rather new, they have not yet been applied extensively to human tissues. The present work was undertaken to determine if there were any unusual amount of depressor substance in (1) carcinomatous tissue and in (2) toxic thyroid tissue.

All specimens were obtained from living patients during a sterile surgical operation. The specimens were extracted in 3 hours or less

4 Miller, C. O., Brazda, F. G., and Elliott, E. C., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 633. Miller, C. O., and Connor, J. A., *Ibid.*, 1933, **30**, 630.

* Work done in part under a grant from the Douglas Smith Foundation.

1 Chang, H. C., and Gaddum, J. H., *J. Physiol.*, 1933, **79**, 255.

2 Dudley, H. W., *J. Physiol.*, 1933, **79**, 249.

3 Euler, U. S., and Gaddum, J. H., *J. Physiol.*, 1931, **72**, 74.

following operation and were kept in a refrigerator at 4°C. in the meantime. The tissue was weighed and placed in 10% trichloracetic acid (2 cc. of acid for each gm. of tissue). It was then cut up in the acid with scissors and left with occasional stirring for several hours. The extract was filtered through paper on a Buchner funnel and the tissue washed with 7% trichloracetic acid. The filtrate was then shaken 3 or 4 times with ether in a separating funnel until it was only faintly acid to congo red paper. It was then concentrated at low pressure at 37°C. until 1 cc. of extract corresponded to about 1 gm. of tissue. This solution was made neutral to congo red solution by titrating with 1-10 N NaOH and then used in the biological test.

In the present work the extracts were tested for effect on dog's and rabbit's blood pressure. The blood pressure was measured by a mercury manometer connected to a cannula in the right carotid artery. The extract was injected by means of a burette connected with a cannula in the left femoral vein of the dogs or the left external jugular vein of the rabbits. The depressor action was compared with that produced by a standard solution of acetylcholine, made up so that 1 cc. corresponded to 1 gamma acetylcholine. If 1 cc. of the unknown extract gave a fall in blood pressure equal to that produced by 1 cc. of the standard solution, then the unknown extract was said to contain 1γ acetylcholine equivalent per cc. An effort was then made to determine whether or not the depressor action was due to acetylcholine or some other substance. According to Chang and Gaddum, acetylcholine is quite inactive when tested on the blood pressure of an atropinized rabbit. As will be shown later in the present work, most of the tissues extracted produced quantitatively as great a fall in blood pressure of a rabbit after atropinization as before. Control injections of pure acetylcholine in these rabbits produced no fall in blood pressure after atropinization. This indicates that in the extracts tested, using the atropinized rabbit's blood pressure as a criterion, acetylcholine was not present in large amounts.

According to Chang and Gaddum, histamine produces little fall in rabbit's blood pressure. In the present studies the tissue extracts were just as active on the blood pressure of rabbits as on that of dogs, both when compared as to absolute dose per unit body weight and as to acetylcholine-equivalent. This indicates that in the extracts tested, using this criterion of Chang and Gaddum, histamine was not present in large amounts.

Euler and Gaddum[3] found large amounts of a substance they

called the "P" substance in certain animal extracts. This substance lowers the arterial blood pressure of the atropinized rabbit. It can be differentiated from adenosine which is stable in alkalis and inhibits the rabbit's intestine. Wilson, Stewart and Harkins,[4] working in Wilkie's laboratory found that the depressor substance in the skin of burned and normal rabbits answered the specifications of the P substance and that acetylcholine was not present in anything but small amounts.

Results. Six human tissues were extracted as follows: (1) Carcinoma of thyroid, woman aged 33 years, basal metabolic rate minus 6 before operation. (2) Quadriceps muscle from amputated leg. (3) Hemorrhagic fluid from aseptic necrosis of large nodule in adenomatous goitre, man aged 60 years, basal metabolic rate plus 21 five days before operation and plus 13 three days before operation. (4) A supposedly normal thyroid gland removed for cardiac disease, man aged 43, basal metabolic rate plus 7 before operation. (5) Pectoralis major muscle from radical mastectomy. (6) Toxic thyroid gland, woman aged 27 years, basal metabolic rate plus 24 just before operation, plus 43 a week previous.

The acetylcholine-equivalents of these extracts are given in Table I. Of the 5 extracts tested before and after atropinization, 2

TABLE I.

The acetylcholine-equivalent of six human tissue extracts. The use of the term acetylcholine equivalent does not imply that the substance tested is actually acetylcholine.

	Before atropine			After atropine		
	No. of animals used in assay		Aver. ac.-equiv.	No. of animals used in assay		Aver. ac.-equiv.
Extract	Dogs	Rabbits	γ per gm.	Dogs	Rabbits	γ per gm.
1. Carcinoma of thyroid	3	3	1.2	2	2	1.1
2. Quadriceps muscle	2	2	2.0	1	1	1.4
3. Thyroid cyst fluid	1	2	0.02	0	0	—
4. Normal thyroid	1	0	8.0	1	0	0.0
5. Pectoralis muscle	2	1	1.8	1	0	0.3
6. Toxic thyroid	2	2	3.6	1	1	5.6

showed only a slight change, 2 were less active and one more active after atropine. For the 2 that were less active after atropine, this conclusion was based on only a single assay in each case. It must be remembered, however, that after atropine, the blood pressure often falls somewhat and the absolute fall produced by an equivalent amount of substance may be less even though the relative fall is as great. The average acetylcholine-equivalent of the 2 normal human

[4] Wilson, W. C., Stewart, C. P., and Harkins, H. N., Depressor Substances in Burned Tissues, to be published.

muscle extracts was 1.9, that of the toxic thyroid was 3.6, the normal thyroid was 8.0, the carcinoma of the thyroid was 1.2, and the fluid from the thyroid cyst was 0.02. These results indicate that in these single instances, there is no unusual amount of depressor substance in toxic thyroid tissue or in carcinomatous tissue.

Controls. In addition to comparing the effects on atropinized and unatropinized dogs and rabbits with those produced by standard acetylcholine solutions, controls were made as follows: (a) 10% trichloracetic acid alone was found to produce a definite depressor action roughly 0.5γ ac.-equiv. per cc. acid. (b) 10% trichloracetic acid was extracted in exactly the same way as the human tissues. This included all steps of the process. Part of this solution was extracted with ether twice, part 4 times and part 6 times. All of these solutions were equally inactive, roughly less than 0.02γ ac.-equiv. per cc. acid. (c) Several of the tissue extracts were assayed without accurate neutralization after extraction and compared with the effects after neutralization to congo red solution and to phenolphthalein. In general these extracts were equally active. (d) Extract No. 5 (pectoralis major muscle) was assayed before and after being passed through a Mandler filter and found to be equally active in both instances.

There are many substances isolated from animal tissues that fall under the classification of depressor substances. These include (a) histamine, (b) acetylcholine, (c) choline and other choline esters, (d) adenosine, (e) P substance, (f) potassium, and (g) R substance.[1] The biological assay used in the present paper indicates that the extracts of human tissues tested contained practically no histamine and very little acetylcholine because they lowered the blood pressure of atropinized rabbits. The various other substances were not definitely excluded, but nothing was found to indicate that the active principle was other than the P substance. The amount of depressor substance in any of the human tissues tested was not as great as that found in certain animal tissues such as the spleen of the horse or ox which Chang and Gaddum found to contain 4 to 30γ ac.-equiv. This amount was said to be actually due to acetylcholine itself. These authors also found 28γ ac.-equiv. in extracts of a human placenta.

Conclusions. In several human tissue extracts, including toxic thyroid tissue and carcinomatous tissue, depressor substances were found not to be present in unusual amounts. The major part of the depressor substance present in these tissue extracts does not act like acetylcholine or histamine when tested for effect on the blood pressure of atropinized rabbits.

7542 C

Rôle of Leucocytes and Serum in Streptococcidal Activity of Blood.

FLOYD BOYS AND F. D. GUNN.

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In testing the defibrinated blood and serum of patients and normal individuals for killing effect against streptococci we found that, while the majority of samples of blood exhibited bactericidal power, the serum alone rarely caused reduction in the number of bacteria. This finding supported the idea that the effective bactericidal agent in whole blood acting against streptococci resides in the cellular elements and confirms the earlier observations of several workers, notably Mackie, Finklestein and Van Rooyden[1] with regard to gram positive cocci in general. Most of the previous work on gram positive bacteria, however, has been done with pneumococci[2] and staphylococci[3] and we considered it worth while to check and extend the work with streptococci.

Leucocyte counts were made at the time of withdrawal of samples of blood and repeated after defibrination was completed. The defibrination was accomplished by rotating the blood with glass beads in an Erlenmeyer flask under sterile precautions. In 6 samples of freshly drawn blood, leucocyte counts of 6930 to 9550 per cu. mm. were obtained and in the same samples after defibrination the leucocyte number was between 5200 and 7160, an average reduction

TABLE I.
Effect of Aging and Chilling on Bactericidal Test.

Case No.		Colony Count-Bc Test				Leucocytes	
		Start	4 hrs.	%	8 hrs.		%
1	Fresh blood	256	6	(—97)	320	6150	
	Chilled "	200	960	(+380)	8000±	4850	(—21)
2	Fresh blood	256	31	(—88)	2080	5300	
	Chilled "	245	8000±	(+3200)	8000±	2435	(—54)
3	Fresh blood	448	8000±	(+1700)	8000±	5940	
	Chilled "	320	8000±	(+2400)	8000±	2825	(—52)
4	Fresh blood	140	5	(—97)	15	6400	
	Chilled "	110	800	(+630)	8000±	2600	(—59)
5	Fresh blood	175	0	(—100)	50	6600	
	Chilled "	160	3	(—98)	576	5350	(—19)

[1] Mackie, J., Finklestein, M. H., and Van Rooyden, C. E., *J. Hyg.*, 1932, **32**, 494.

[2] Robertson, O. H., and Sia, R. H. P., *J. Exp. Med.*, 1924, **39**, 219.

[3] Thalhimer, W., and Colwell, C., *J. Lab. and Clin. Med.*, 1929, **24**, 441.

of 23%. This reduction was not sufficient to produce an appreciable alteration of the bactericidal power of the blood as proved by previous experiments. After rotatory agitation for periods of 4 hours without the addition of bacteria, however, the reduction in number of intact leucocytes was from 70% to 90%.

The effect of standing and refrigeration is illustrated in Table I, which gives the comparative colony counts from bactericidal tests made on fresh samples and samples allowed to stand in the refrigerator overnight. These were incubated with the test culture for periods of 4 and 8 hours in the agitating machine. In each blood sample that exhibited definite killing action when fresh, there was marked reduction of bactericidal power after refrigeration. In these samples the degree of reduction in number of leucocytes varied between 19% and 59%. It is obvious that the numerical reduction in leucocytes is not the most important factor. The phagocytic activity of the intact leucocytes must have been seriously impaired by such treatment.

Next a small series of tests was made to determine whether or not some of the inhibitory effect of the blood would remain after complete removal of the leucocytes. Four samples of defibrinated blood from different patients were divided into 2 portions and one part centrifuged at low speed to throw down the formed elements. The buffy coat was removed as completely as possible with sterile pipettes and parallel tests were run on these samples and on the complete defibrinated blood as controls. The results (Table II) indicate

TABLE II.
Effect of Removal of Leucocytes.

Case	Colony Count (Control)			Colony Count (Leucocytes removed)		
No.	Start	4 hrs.	8 hrs.	Start	4 hrs.	8 hrs.
1	2880	8000±	8000±	2180	8000±	8000±
2	2240	37	86	2200	8000±	8000±
3	190	0	0	210	8000±	8000±
4	200	1	160	215	8000±	8000±

that after the more or less complete removal of leucocytes, blood no longer inhibits the growth of streptococci. In case 1, however, the blood failed to show any inhibitory effect even in the control sample.

The apparent exhaustion of bactericidal power after about 4 hours of incubation and the demonstration of marked reduction of leucocytes in mixtures that had been incubated for a few hours with rotatory agitation led us to suspect that the loss of killing power was due merely to exhaustion and disintegration of the leucocytes. If true, an addition of fresh leucocytes should restore the exhausted

factor. This was found to be the case in each of 5 samples from different patients where the blood was fortified by the addition of leucocytes at the end of 4 hours of incubation. The volume of leucocyte suspension added to each tube was equal approximately to that at the beginning of the test. (Table III.)

TABLE III.
Bactericidal Power after Fortification with Leucocytes.

Case	Control			Fortified at 4 hours		
No.	Start	4 hrs.	8 hrs.	Start	4 hrs.	8 hrs.
1	520	8000±	8000±	560	8000±	1216
2	80	160	4000±	90	175	115
3	2880	8000±	8000±	2900	8000±	1940
4	240	19	160	250	28	15
5	152	4	200	160	7	30

In a similar group of cases the blood samples were each divided into 2 equal portions. One was run as usual as the control and the other was subjected to rotatory agitation in the incubator for 4 hours before the suspension of bacteria was added. The killing effect of the blood was completely destroyed in 3 samples and seriously impaired in the other 2 as a result of the preliminary incubation (Table IV).

TABLE IV.
Reduction of Bactericidal Power after Preliminary Incubation.

Case	Control			4 hrs. Preliminary Incubation		
No.	Start	4 hrs.	8 hrs.	Start	4 hrs.	8 hrs.
1	288	0	58	300	728	8000±
2	432	0	0	400	368	124
3	800	13	62	780	8000±	8000±
4	820	125	548	800	8000±	8000±
5	592	11	0	610	8000±	8000±

The mode of destruction of the bacteria and the rate at which they were removed were determined by the examination of stained films made from the mixtures at intervals during the tests. For this experiment suspensions containing about 200 million bacteria per cc. were used. Blood films were made in the usual manner from a drop removed by sterile capillary pipette and stained with Wright's blood stain. The relative numbers of polymorphonuclear cells containing bacteria and those showing no bacteria in their cytoplasm at the different intervals are recorded in Table V. Toward the end of the 4-hour period the leucocytes were so few that only 10 or 15 leucocytes were found by systematic examination of the entire film. In each case the majority of the leucocytes contained phagocytosed

TABLE V.
Rate of Phagocytosis in Bactericidal Tests.

Case No.		Start	½	1	1½	2	3	4	8 hrs.
1	Pmn with bac.	3	6	12	13	25	15	10	
	No intrac. bac.	22	19	13	2	0	0	0	
	Col. in Bc test	100						20	64
2	Pmn with bac.	5	8	18	25	25	18	15	
	No intrac. bac.	20	17	7	0	0	0	0	
	Col. in Bc test	80						160	6000±
3	Pmn with bac	10	23	25	25	25	20	10	
	No intrac. bac.	15	2	0	0	0	0	0	
	Col. in Bc test	150						1	1
4	Pmn with bac.	4	11	15	24	25	15	15	
	No intrac. bac.	21	14	10	1	0	0	0	
	Col. in Bc test	190						160	640
5	Pmn with bac.	12	40	44	46	50	50	50	
	No intrac. bac.	38	10	6	4	0	0	0	
	Col. in Bc test	200						1	1

cocci by the end of one or one and one-half hour and in 2 hours all of the cells contained bacteria in various stages of disintegration. No significant differences in rate of phagocytosis were observed in those blood samples exhibiting marked inhibitory power in the bactericidal tests and those showing less power.

In preliminary experiments it was found that while the presence of serum was necessary for the obtaining of bactericidal effects, dilutions up to 1-25 were apparently as effective as undiluted serum. The agglutinating titer had been found to bear no constant relationship to the bactericidal power of whole blood, defibrinated blood or serum. The effect of varying complement concentration remained to be determined. In 10 cases the complement titer and bactericidal power of the defibrinated blood were determined on the

TABLE VI.
Bactericidal Power and Complement Titer.

Case No.	Serum Dilution	Beginning Lysis cc.	Complete Lysis cc.	Colony Count in Bc Test		
				Start	4 hrs.	8 hrs.
1	1-50	.2	0.5	160	2	368
2	1-50	.1	0.2	340	1420	8000±
3	1-50	.2	1.0	256	31	2080
4	1-25	.5	0.8	240	0	0
5	1-25		0.3	288	23	380
6	1-50	.5	1.0	240	8000±	8000±
7	1-50	.5	1.0	240	3	450
8	1-50	.9	1.0	152	4	200
9	1-25		0.5	250	10	50
10	1-50	.7	1.0	192	0	0

same sample (Table VI). No correlation was observed. One of the two samples of blood showing the least bactericidal power, contained the highest concentration of complement and the 2 samples having the greatest killing power yielded very low complement titer.

Finally the thermostability of the active factor was tested by removing the serum as completely as possible from samples of defibrinated blood after centrifugation and heating it at 55°C. for 30 minutes. The inactivated serum was then restored to the cells from which it had been removed and bactericidal tests run. For controls we used a part of the same sample, subjected to the same treatment with the exception that the serum was not heated. In a few cases a part of the serum was heated to 68°, returned to the cells and then tested for killing effect. Similar tests were made with serum alone after heating at 55° or 68° for 30 minutes and with unheated controls. In none of these cases was there any evidence that the bactericidal property was impaired by the heating of the serum.

Conclusions. The power of defibrinated blood to inhibit the growth of or destroy streptococci is dependent upon the presence of surviving leucocytes and is roughly proportional to the number of leucocytes, other factors being equal. A minimal number of leucocytes is necessary for the demonstration of the bactericidal effect. The bacteria are removed by phagocytosis and finally destroyed by intracellular lysis. Complement is not necessary for the bactericidal action of defibrinated blood or serum. The bactericidal element in defibrinated blood which is effective against streptococci is not destroyed by heating the separated serum at 55°C. for 30 minutes. The results of a small number of tests suggest that the heating of the serum even at 68°C. does not impair the bactericidal effect of the restored defibrinated blood.

7543 P

Development of Gastric Ulcers and Decrease in Reducing Power of Adrenals Following Injection of Bile Salts.

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Sellards[1] reported the development of acute gastric ulcers in guinea pigs following intraperitoneal bile salt injection. His ob-

servations have been confirmed and extended by Tashiro and coworkers.[2] We injected approximately 40 pigs with varying amounts of bile salts (Fairchilds). The expected individual variations in susceptibility were observed, but injections of 0.1 gm. or more generally caused death and extensive ulceration of the gastric mucosa. The latter was usually so marked that the site of the ulcers could be plainly observed from the outer surface of the stomach, appearing as thin, semi-transparent areas. In 3 animals perforation occurred, gastric contents being found in the peritoneal cavity at autopsy.

Szent-Gyorgyi[3] reported the darkening of the adrenal cortex when subjected to a silver nitrate solution. Harris and Ray[4] and Siehrs and Miller[5] report that this does not occur in guinea pigs on a scorbutic diet. When we attempted to stain, with silver nitrate, the adrenals of pigs previously injected with bile salts, darkening was slight or absent. Adrenals of apparently normal pigs may, however, not stain with silver nitrate. Gough and Zilva[6] report that in pigs given 10 cc. of decitrated lemon juice daily for a period of 3 months the adrenals did not stain, although at autopsy no abnormalities were observed. We kept 12 pigs on a diet of oats, an occasional carrot, and 2 cc. of orange juice daily for one week. The cortex of the adrenals did not stain with silver nitrate. The absence of this staining reaction may merely indicate an insufficient excess of vitamin C in the diet to allow for its deposition in the adrenal. Therefore, in the remaining experiments, a diet abundant in vitamin C was administered, the pigs receiving oats, fresh carrots and cabbage, and, in addition, 4 cc. of orange juice daily for a period of 3 weeks prior to injection. The adrenals of control animals stained almost totally black with 0.4% silver nitrate solution when exposed for 3 minutes to a 115 watt blue mazda lamp at a distance of approximately 8 inches. The adrenals of animals injected with bile salts also showed some reduction under similar treatment, but in practically all cases the extent of darkening was distinctly less than in the controls and tended more toward a brown than to the black of the controls. The decrease in reducing power was also confirmed by iodine titration, which, although not specific for vitamin

[1] Sellards, A. W., *Arch. Int. Med.*, 1909, **4**, 502.

[2] Tashiro, *et al.*, *Med. Bull. Univ. Cincinnati*, 1931, **6**, 110, 124, 130, 134, 144.

[3] Szent-Gyorgyi, A., *Biochem. J.*, 1928, **22**, 1387.

[4] Harris, L. J., and Ray, S. N., *Biochem. J.*, 1933, **27**, 303.

[5] Siehrs, A. E., and Miller, C. O., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 696.

[6] Gough, J., and Zilva, S. S., *Biochem. J.*, 1933, **27**, 1279.

C, should reveal any marked decrease in this substance. Unfortunately at the time these experiments were conducted, we did not have available 2,6-dichlorophenolindophenol, which, according to recent work, exhibits considerable specificity for ascorbic acid.[7] The technique consisted in grinding the whole adrenal under 10% trichloracetic acid, filtering, and washing the residue, followed by titration of the filtrate with 0.002 N iodine, using starch as the indicator. For titration the left adrenal was generally employed, the right having been used for silver nitrate staining. From Table I it

TABLE I.
Iodine Titration of Adrenals Following Bile Salt Injection.

Sex	Wt. gm.	Dose bile salts	Time for death	Wt. adrenal gm.	cc. I_2	cc. I_2/gm. adrenal
M	234	control	killed	.065	.65	10.0
M	226	"	"	.065	.61	9.4
F	187	"	"	.034	.35	10.3
F	251	"	"	.074	.75	10.1
M	265	"	"	.042	.62	14.8
M	291	"	"	.047	.50	10.6
F	254	"	"	.053	.77	14.5
M	188	.05 g.	4¼ hrs.	.061	.40	6.6
M	167	.10	5¼	.073	.61	8.3
M	207	.10	2¼	.075	.16	2.1
M	200	.15	1¼	.059	.50	8.5
F	187	.10	4¼	.038	.40	10.5
F	175	.15	1¼	.050	.27	5.4
F	205	.15	2½	.067	.20	3.0
F	—	.15	2½	.076	.48	6.3
M	312	.05	26	.066	.53	8.0
M	286	.10	4	.064	.63	9.8
M	304	.15	2	.075	.57	7.6
F	304	.10	4	.052	.38	7.3
F	292	.10	4½	.082	.69	8.4
F	290	.15	2	.067	.46	6.9
M	332	.10 initial .05 23 hrs. .05 27 " .10 29 "	30½	.067	.56	8.3
F	294	.05 initial .05 23 hrs. .05 26 "	31	.102	.80	7.8

Average cc. iodine/gm. adrenal controls = 11.4
injected pigs = 7.2

will be observed that injected animals showed a decreased iodine titration.

A series of 26 rats was also injected with bile salts. Doses comparable with those used with guinea pigs usually caused death within 24 hours, but in no case was ulceration similar to that produced in guinea pigs observed. Occasionally slight, superficial

[7] Birch, T. W., Harris, L. J., and Ray, S. N., *Biochem. J.*, 1933, **27**, 590.

erosions were seen, but nothing comparable with the deep, extensive ulcers which developed in the guinea pigs was observed. When the rats' adrenals were stained with silver nitrate, the darkening was immediate and marked. At present we are unable to say definitely whether this resistance of the rat to ulcer production through bile salt injection is due to their ability to synthesize vitamin C or to some other species difference.

7544 P

Differential Reduction of Methylene Blue by Living Organisms.

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With the oxidized dye and with a leucobase prepared by adding small amounts of HCl and $Na_2S_2O_3$ to dye solutions axial differentials or gradients in rate of reduction of dye have been observed in various unicellular and multicellular organisms. *Paramecium* is able to reduce methylene blue in mixtures of culture fluid and dye exposed to air, provided the animals are numerous in proportion to volume of fluid or gather in aggregations and decrease oxygen locally, or provided other organisms which take up oxygen are present, but reduces more rapidly in sealed preparations with small amounts of fluid. With high oxygen content of solutions the anterior ectoplasm stains more rapidly than other parts. In low concentrations of dye with somewhat lower oxygen content permitting some reduction stain appears first in the deepest part of the posterior entoplasm, extends anteriorly in the entoplasm and the ectoplasm does not stain or stains more slowly, also from posterior to anterior, except for the extreme posterior tip which often stains less rapidly than adjoining regions. Apparently rate of staining under these conditions varies inversely as reducing power of different regions. In stained but uninjured animals reduction first becomes evident in the ectoplasm of the anterior end and progresses posteriorly, somewhat more rapidly along the peristome than on the aboral side and the extreme posterior tip of some individuals shows early reduction. In high concentrations the anterior ectoplasm stains more rapidly and more deeply than other regions at first and with sufficient staining it is injured and its reducing power is decreased or lost while more posterior regions are still able to reduce the dye in low oxygen.

Other ciliates examined, *Frontonia, Spirostomum, Dileptus,* show differentials in staining and reduction essentially similar to those of *Paramecium,* but with different ranges of concentration and different degrees of decrease in oxygen. The animals and other material from the infusion decrease the oxygen sufficiently to permit more or less reduction in *Frontonia* and *Dileptus* in open preparations, while *Spirostomum* requires several hours in sealed preparation for complete reduction after slight staining.

In *Hydra* tentacles stain first with concentrations used thus far and if staining is not carried to the point of injury each tentacle shows a basipetal reduction gradient in low oxygen. With further staining before reduction loss of reducing power occurs first at the tentacle tip and progresses basipetally as staining proceeds and cytolysis follows basipetally. In the body ectoderm reduction occurs most rapidly in the hypostome region and progresses basipetally, provided staining has not progressed to the point of injury and loss of reducing power. The basal stalk region of *Pelmatohydra* reduces more rapidly than the body after light staining, but is also more susceptible than the body and injury and loss of reducing power occur with comparatively little staining. In *Stenostomum* chains of zooids the head regions of the developing zooids beyond a certain stage of development reduce the dye more rapidly than more posterior levels with light staining, but are more susceptible to loss of reducing power after deeper staining. The ventral body wall also reduces more rapidly than the dorsal. In the microdrilous oligochetes, *Tubifex* and *Nais* the body wall of the anterior region and the posterior growing region consisting of a large number of developing segments do not stain or stain less rapidly than the middle region and the ventral body wall does not stain or stains less rapidly than the dorsal in low concentrations of dye in the oxygen content of the cultures of decaying vegetation, bacteria, protozoa, etc., in which the animals are maintained in the laboratory. After staining up to a certain point anterior and posterior regions reduce more rapidly than the middle in low oxygen and the ventral body wall reduces more rapidly than the dorsal. With high dye concentrations the anterior end stains more deeply than the middle and its reducing power is decreased or lost. The posterior region is so susceptible that it is usually killed before it becomes very deeply stained.

Concentrations used in these experiments range from 1/5000 to 1/300000 methylene blue. Since the leucobase stains and injures some forms (*e. g., Paramecium*) much more rapidly than the oxidized dye the range of concentrations for certain results is lower

than with the latter. The dye is more toxic in light, even the light of the microscope condenser, than in darkness.

That the results described are due to reduction, not to diffusion outward of the dye is shown by the fact that after reduction the color returns within a few seconds with increase in oxygen content of the fluid. It may also be noted that in all cases the reduction gradient in animals not irreversibly injured is essentially identical with the gradient of susceptibility to a large number of chemical and physical agents in gradually lethal concentration or dosage.

The anteroposterior reduction gradient in *Paramecium* was noted in an earlier paper.[1] Recently Roskin and Semenoff,[2] using a leucobase only, have concluded from the observed course of reduction that oxidation occurs most rapidly in the posterior region of *Paramecium*. Their results can be duplicated by following their procedure but this procedure results in deeper staining of the anterior ectoplasm and less rapid or no reduction there while more posterior regions are still able to reduce, consequently they have failed to observe the normal anteroposterior reduction gradient.

7545 C

Effect of 1-2-4 Dinitrophenol on Cellular Respiration.

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From the Lasker Foundation for Medical Research and the Department of Medicine, the University of Chicago.

Numerous investigators (Plantefol,[1] Field, Martin and Field,[2] Ehrenfest and Ronzoni[3]) have reported that 1-2-4 dinitrophenol added *in vitro* to plants, yeast cells and frog tissues increases their respiration. This increase seems to be associated with an increased aerobic fermentation in the case of yeast cells (Cutting and Tain-

[1] Child, C. M., and Deviney, E., *J. Exp. Zool.*, 1926, **43**, 257.

[2] Roskin, G., und Semenoff, W., *Z. f. Zellforschung u. mikr. Anat.*, 1933, **19**, 150.

* Rockefeller Foundation Fellow.

[1] Plantefol, L., *Ann. Physiol. Physicochim. Biol.*, 1933, **8**, 127.

[2] Field, J., 2nd, Martin, A. W., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 56.

[3] Ehrenfest, E., and Ronzoni, E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 318.

[4] Cutting, W. C., and Tainter, M. L., *J. Pharm. and Exp. Ther.*, 1933, **48**, 410.

ter[4]) and an acceleration of anaerobic lactate production in the case of frog tissues (Ehrenfest and Ronzoni).

The experiments here reported were performed in an attempt to determine the mechanism of this rise in cellular respiration. The oxygen consumption was measured with the Warburg apparatus. The yeast (baker's) was suspended in 0.066 M phosphates; the frog tissues, in frog Ringer's, buffered with phosphates to pH 7.46; the goose red cells in 0.9% NaCl buffered with phosphates to pH 7.46. All these solutions contained 0.2% glucose. Gonococci were suspended in 0.9% NaCl and buffered with phosphates to pH 7.01 and pH 6.0.

Field, Martin and Field's contention that dinitrophenol is active only in its undissociated form is not supported by the findings of Ehrenfest and Ronzoni, nor by our own experiments. Dinitrophenol increased the respiration of yeast at pH 6.64 (where the concentration of the undissociated form was only 0.0158 mg. per liter), and the respiration of frog tissues and goose red cells at pH 7.46 (where dinitrophenol is practically wholly dissociated) (Table I).

TABLE I.
Effect of 1-2-4 Dinitrophenol on Respiration of Tissues and Cells.

Tissue	Concentration of Dinitrophenol mg./L.	O_2 Consumption		% Increase
		Before D.N.P. c.mm./hr.	After D.N.P. c.mm./hr.	
Yeast	50	121.8	151.6	20
pH = 6.64		136.5	156.3	13
T = 25°		140.5	155.1	9
		124.6	157.2	21
		137.3	184.7	26
		114.0	174.5	35
Frog Kidney	5	21.2	37.8	44
pH = 7.46		4.6	7.2	36
T = 30°		12.0	20.4	41
		22.6	34.2	34
Frog Liver	5	15.6	21.4	27
pH = 7.46		15.8	24.6	36
T = 30°		24.6	34.0	28
		11.0	12.8	14
Goose Red Cells	7	73.6	113.5	54
pH = 7.46		76.3	112.0	47
T = 37°		77.7	119.3	54
	13	75.6	140.0	85
		82.3	140.0	70

The increase in cellular respiration observed after the addition of dinitrophenol seems not to be due to a direct oxidizing action of this compound on the oxidizable substrates. Thus dinitrophenol did not oxidize lactate activated by α-hydroxyoxidase of gonococci, an oxida-

tion readily catalyzed by reversible dyes (Barron and Hastings[5]) nor did it oxidize linseed oil, an oxidation catalyzed by hemin (Robinson[6]). Furthermore, when cellular respiration was inhibited by the addition of specific inhibitors of the oxidizing enzymes, KCN and CO, there was no increase of respiration after addition of dinitrophenol. Indeed some decrease of respiration was observed upon the addition of dinitrophenol to cyanide-treated goose red cells. These experiments were performed with frog kidney and liver, and goose red cells in the case of cyanide (0.002 M) and frog kidney and yeast in the case of CO (Table II). We may add that the formation of the potassium salt of metapurpuric acid by the action of KCN on aqueous solutions of dinitrophenol, occurs only in alkaline reaction and at 60°. At 38° and pH 7.46 (the pH of our experiments) such a reaction does not occur.

Dinitrophenol showed no accelerating influence on oxidations produced by gonococci. As oxidizable substrates, glucose, lactate, and pyruvate were used (Table III).

TABLE II.
Effect of 1-2-4 Dinitrophenol on Cells and Tissues after Inhibition of Respiration by KCN and CO.

Tissue	Inhibitor	O_2 Consumption	
		Before D.N.P.	After D.N.P.
		c.mm./30 min.	c.mm./30 min.
Frog Kidney	KCN	2.5	1.0
		2.5	3.1
		5.0	4.9
		4.6	4.1
		3.3	2.6
Frog Liver	KCN	2.9	1.7
		7.3	2.5
		6.1	3.7
		6.4	6.0
		4.0	0.7
Goose Red Cells	KCN	17.9	10.8
		19.7	9.6
		31.1	6.8
		20.2	5.2
Frog Kidney	$CO:O_2$ (96.5:3.5)	1.1	0.4
		0.4	0.4
		1.4	1.1
		1.3	0.9
Yeast	$CO:O_2$ (90:10)	64.0	66.0
		66.2	64.6
		52.2	55.0
		64.2	65.2

[5] Barron, E. S. G., and Hastings, A. B., *J. Biol. Chem.*, 1933, **100**, 155.
[6] Robinson, M. E., *Biochem. J.*, 1924, **18**, 255.

TABLE III.
Dinitrophenol and Oxidations Produced by Gonococci.

Substrate	O_2 Consumption in 30 Minutes	
	Without D.N.P.	With D.N.P.
Glucose	234.8	234.8
Lactate	48.2	44.0
Pyruvate	130.0	91.9

Conclusion. Since dinitrophenol is unable to oxidize such a labile compound as lactate activated by α-hydroxyoxidase; is without effect when the respiration of cells and tissues has been inhibited by cyanide or carbon monoxide; and has no action on the respiration of certain bacteria, where the complicated controlling mechanisms present in highly organized cells are absent, it is concluded that the increase in respiration produced by dinitrophenol is not due to direct oxidation of the oxidizable substrates. It is suggested that dinitrophenol acts by combining with some of the substances acting as agents for the control of the speed of cellular oxidations, thus increasing the activity of the oxidizing enzymes.

7546 P

Effect of Histamine and Alcohol on Acid Secretion of Stomach of Postoperative Cases.

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In order to stimulate the secretion of acid by the stomach of postoperative surgical cases, the action of histamine and alcohol was tested. In 10 cases a Rehfuss tube was inserted through the nose

TABLE I.
Histamine Tests.

No.	Control		15 min.		30 min.		45 min.	
	Free	Total	Free	Total	Free	Total	Free	Total
1	0	11	12	36	82	102	73	95
2	0	—	30	48	68	85	55	75
3	0	—	27	51	90	112	80	105
4	0	—	27	51	90	112	80	105
5	30	55	32	54	72	89	65	87
6	0	—	0	—	0	—	0	—
7	0	—	0	—	—	—	60	81
8	0	—	20	38	89	104	119	129
9	0	—	0	—	0	—	0	—
10	0	—	112	134	124	142	135	155

before complete recovery from the anesthesia and 2 to 5 hours later, 0.5 or 1.0 mg. histamine given hypodermically.

The results of these tests indicate that in many cases at least the impaired acid secretion may be readily remedied by histamine. The flow in 6 of the 10 cases was profuse and contained a high acid content. The reaction did not seem to be dependent on the type of operation or anesthetic as some of the minor operations failed to secrete while major operative cases often yielded high acid contents. One of those which failed to secrete acid had very large amounts of bile in the stomach which might have masked it but the other 2 did not.

Five similar tests were made with alcohol. The usual technique of the alcohol test meal was carried out, 50 cc. sauterne 15% alcohol being injected through the nasal tube.

TABLE II.
Alcohol Tests.

No.	Control		15 min.		30 min.		45 min.	
	Free	Total	Free	Total	Free	Total	Free	Total
1	0	—	40	63	46	70	35	53
2	0	—	0	—	0	—	0	—
3	0	—	0	—	0	—	0	—
4	0	—	56	83	110	112	56	72
5	0	—	0	—	0	—	0	—

Thus while but 2 of the 5 responded at all, in these two the acid reached very high titres.

It is clear, therefore, that in some cases at least the stomach of the usual postoperative case, in which achlorhydria is the rule, is capable of stimulation to secrete hydrochloric acid. There was obvious clinical benefit from the procedure. None of the patients vomited and none were nauseated. All were promptly given large amounts of solid food and appeared to have no discomfort. The contrast with the usual surgical patient at this stage of his convalescence was very marked.

Conclusion. Histamine and alcohol have their usual action of stimulating gastric secretion in the postoperative surgical patient. Their administration is clinically beneficial.

7547 P

Studies on Acholic Cachexia. V. Pathological Changes.*

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Many observers have reported extensive pathological changes in the organs of animals suffering from cachexia cholipriva. These have in general consisted of 3 processes; (1) degenerative changes in the liver, pancreas, heart and skeletal muscles, (2) osteoporosis, and (3) hepatitis, due to ascending infection from the cannula. Whether the first 2 types of changes are due to lack of bile in the system or due to ascending infection of the liver has not been clearly understood.

Our experience with bile fistulas made by various methods has been that ascending cholangitis was nearly always present, in some cases being of a severe type. The bile canaliculi were surrounded by rosettes of infiltrating cells and degeneration of the lobules of the liver was marked. However, with fistula made by the Dragstedt cannula, this has not occurred. The liver showed no signs of infection. The bile ducts were clear, no bile thrombi were found and even when the fistula had persisted for 5 months there were absolutely no infiltrations of leucocytes about the bile ducts, and no fibrosis. In these cases there was a corresponding absence of other signs of liver damage. Central necrosis, ordinarily seen over large areas did not occur. The cells stained clearly and contained no vacuoles or fat droplets. Iron stains showed that a small amount of pigments was hemosiderin and not bile pigment. This was also seen in the spleen but was not of a high degree. Normally, the dog has considerable pigment deposits of this type in the spleen and liver. Similarly, in spite of the extreme emaciation of the animals the other viscera were normal. The myocardium showed no waxy degeneration nor did the skeletal muscle. The intestinal and gastric mucosa was normal. Konjetzky gastritis was not present as has been reported previously, there being no eosinophiles in the submucous layers. The adrenals were normal, except for the total absence of any fat droplets, or lipoid.

Not only in the series here reported, but in an extensive former series of bile fistulas, has osteoporosis been conspicuous by its ab-

* Work done in part under the Jessie Horton Koessler Fellowship of the Institute of Medicine.

sence. The literature on this subject is highly contradictory. Since Pavlov first reported osteoporosis with biliary fistulas, Looser[1] has found it to occur also with pancreatic and intestinal fistulas. Seidel[2] reports similar findings. In some reports the bone absorption was by means of osteoclasts and in others no lacunar absorption was noted although porosis was present. Modern views are distinctly against the humoral absorption of bone, the so-called halisteresis. Klinke[3] believed the bone atrophy was simply an accompaniment of general cachexia, and it is true that in most cases reported clinically this was extreme as in the recent paper of Wangensteen.[4] At any rate in a considerable series of fistulae we have not observed osteoporosis, as estimated either by X-ray or by histological demonstration of lacunar absorption. Klinke estimates that about two-thirds as much calcium is lost in the bile as in the urine. This loss hardly seems excessive. More important is the recent demonstration that calcium may be absorbed from the intestinal tract in the absence of bile. The obvious explanation seems to be that an infected bile fistula produces a marked acidosis. Our dogs, not having an acidosis, had no porosis.

Conclusion. 1. In uninfected bile fistulas no degenerative changes in the liver occur nor does osteoporosis.

7548 P

Studies on Acholic Cachexia. VI. Bile Acid Factor.*

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The classic studies of Whipple and his co-workers in this field have elucidated clearly the general problems of bile acid metabolism. Our work is in general confirmatory of these, but certain other factors enter into the situation when the cachexia becomes extreme. In no case has any ingestion of bile been permitted in our experiments and the analysis of the bile in the later cachetic stages brings out new points.

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[1] Looser, *Verhandl. deutsche Path. Ges.*, 1907, **11**, 291.

[2] Seidel, *Munch. Med. Wochschr.*, 1910, **57**, 2034.

[3] Klinke, *Klin. Woch.*, 1928, **1**, 385.

[4] Wangensteen, *J. Am. Med. Assn.*, 1929, **93**, 1199.

* Work done in part under the Jessie Horton Koessler Fellowship of the Institute of Medicine.

As has been the experience of all previous workers, the total output of bile acids varies over a wide normal range. It varies not only with the individual dog, the diet, the fluid intake and the general condition of the animal, but other unknown factors evidently cause even wider fluctuations. Careful examination of our records often fails to give any explanation of fluctuations amounting to 200-300%. In general the amounts expressed in milligrams per kilo per day were considerably less than those given by Whipple. He estimates that a normal 10 kilo dog keeps about 7-8 gm. of bile salts per kilo in circulation, and that a fistula dog puts out 80-130 mg. per kilo per day. As can be seen from our tables our dogs put out but 20-40 mg. per kilo per day in the earlier stages, and when the cachexia became extreme often excreted only a small fraction of that amount.

TABLE I.

	Average Daily Mg. B.A. for Period			
Postoperative	Dog No. 958 Wt. 19.5	Dog No. 959 Wt. 17.5	Dog No. 940 Wt. 18.8	Dog No. 960 Wt. 17.5
Days				
6	223	210	1308	375
10	327	210	1308	375
15	398	528	1308	375
19	398	432	554	991
23	184	342	554	135
27	612	212	665	135
31	604	450	914	258
36	609	466	1237	288
40	383	713	1375	853
44	418	339	1137	583
48	401	211	646	295
56	580	393	1208	441
64	460	422	1096	551
72	540	422	976	197
99	622	422	278	609
114	105	192	61	—
130	105	194	61	—
150	402	194	—	—
167	602	128	—	—

The relation of the condition of the dog to the amount of bile salts excreted is not a direct one. Two of the 4 animals here reported excreted even more freely during the terminal stages. In our experience the opposite has more often been true. It is clear, however, that, if a very high excretion may in some cases be maintained right up to death from inanition, it is logical to assume that lack of bile salts in the body is not a major causative factor in the cachexia.

Both in these and in previous experiments, it has become evident that diet is not the only factor in regulating the bile salt output in cachexia. If as Whipple says the basic endogenous bile salts from

tissue waste amount to 30-40 mg. per kilo per day and the rest is dietary, this mechanism does not necessarily work in the later cachectic stages. While in some cases the increase of meat in the diet may markedly increase the amounts of bile salts excreted, in others (dogs No. 940 and 959) the opposite occurs. It is well known that a meat diet hastens the exitus of bile fistula animals and in many cases such as this the added intoxication resulting from the meat seems to bring about a closing down of the excretory mechanism.

Conclusion. Acholic cachexia is not due to lack of bile salts in the body.

7549 P

Studies on Acholic Cachexia. VII. Effect of Viosterol.*

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From the Department of Surgery, The University of Chicago.

The rapid loss of weight, anemia and other changes which may occur in biliary fistula have recently been attributed to lack of ability to absorb fat-soluble vitamin. Takasu[1] has postulated that lack of bile salts in the intestinal tract prevents absorption of ergosterol in the same manner that fat digestion is impaired or stopped. Murakamis[2] reports that the excretion of bile acids is quadrupled by a single cubic centimeter of irradiated ergosterol, given subcutaneously. Others have found that vitamin administration had a markedly beneficial effect on the anemia and also prevented the bone changes.

Our experiences do not substantiate any of these conclusions. As shown in a previous article, the anemia did not seem an important factor. It was not profound and occurred mostly as a terminal phenomenon. Also the bone changes have not occurred in animals who did not have an ascending hepatitis. In 2 animals in whom frequent blood counts were made there was no effect from the administration of ½ cc. viosterol (250 D per cc.). In these experiments the viosterol was given about the third month and the fall in the hemoglobin and red count was more rapid after its administration

* Work done in part under the Jessie Horton Koessler Fellowship of the Institute of Medicine.

[1] Takasu, M., *Deutsche Z. f. Chir.*, 1930, **224**, 240.

[2] Murakamis, R., *J. Biol. Chem.*, 1928, **9**, 321.

than before. As to the bone changes, much emphasis has been put by some workers on the lack of rise in blood calcium after viosterol administration to bile fistula dogs. The well known tolerance of the dog to viosterol is here overlooked. Its calcium threshold is low and viosterol injection even in very large doses does not affect the blood calcium even of the normal dog, so it would hardly be expected to act any better in the presence of bile fistula.

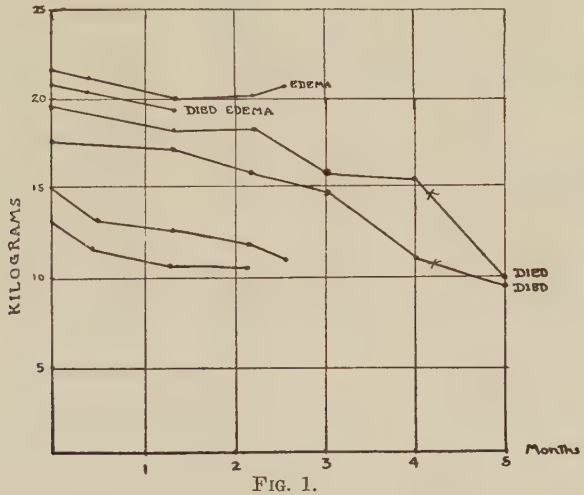

FIG. 1.

Effect of Viosterol in Bile Fistula Dogs.
4 short experiments had viosterol 250 D 1 cc. every 4 days.
2 long experiments had viosterol 250 D ½ cc. daily from point marked X.

Much of the previous work has been done on dogs which were allowed to lick their fistulae and such animals may be kept in good health for very long periods. If this is rigidly guarded against as in our experiments, in quite an extensive series, the loss of weight has been irregular and at the rate of about 1-1.5 kilos a month. It is quite clear from the accompanying table that administration of viosterol even in very large doses has no effect whatsoever on the progress of the cachexia nor does it appear to save the life of any of the animals.

The completeness of the biliary fistula may be the cause of the apparent differences in our experiences from those of others. It is quite conceivable that an animal which gets minimal but adequate amounts of bile by mouth (5 cc. daily according to Whipple) is in a different condition from those having none. In the former condition viosterol may exert a profound effect which is absent in the latter.

Conclusion. Viosterol does not exert a favorable influence on dogs with complete biliary fistulae.

7550 P

Influence of Variations of O_2 and CO_2 Tension in Inspired Air Upon Hearing.

ERNST GELLHORN AND IRWIN SPIESMAN.

From the Department of Physiology, College of Medicine, University of Illinois.

Although tests made on aviators and other observations at high altitudes indicate that O_2-lack causes sensory disturbances,[1] particularly in vision, no systematic study seems to have been made in which the influence of various O_2- and CO_2-tensions was investigated in regard to sensory functions. Such a study seems to be of particular interest since the importance of these factors for various reflexes is well known. The present paper is based upon 96 audiometer experiments carried out on 6 thoroughly trained subjects. The administration of various O_2-, N_2- and CO_2-air mixtures, which were inhaled from several large Douglas bags, was preceded and followed by control periods in which the threshold for a certain sound was determined in intervals varying between 1 and 4 minutes. Long control series extending over several hours were also carried out in order to determine the spontaneously occurring variations in threshold. The observation room was nearly sound proof.

In 37 experiments the influence of breathing CO_2-air mixtures (2%-8.4%) during 5-22 minutes was studied upon the threshold of C 128, C 2048, and C 4096 cycles per second. It was found that a distinct hearing loss occurred during CO_2 breathing at and above 3% CO_2. Depending on the CO_2 concentration and the duration of the breathing period, the recovery after the end of the CO_2-period varies somewhat, but in general it was found that in less than 15 minutes the threshold was the same as before the experiment.

Contrary to expectation, similar losses in auditory acuity were obtained in 26 experiments in which the CO_2 tension of the blood was lowered by means of voluntary hyperpnea which was carried out for 3-6 minutes. The threshold determined immediately after the hyperpnea period during which apnea obtained was considerably

[1] McFarland, R. A., *Arch. Psychol.*, 1932, **145**, 1.

higher and a gradual return to normal was observed during the next 10-15 minutes.

In a third group of 33 experiments 7.5%-15.8% O_2 was inhaled for 8-30 minutes. Here again it was found that during the period of O_2-lack a decrease in hearing occurred. The recovery period, after readmission of air, depended greatly upon the degree of O_2-lack produced during the experimental period. If 10% or less O_2 was inhaled for 15-30 minutes a decrease in hearing persisted for considerable periods of time, in some cases for several hours. During this time no other symptoms were present. The administration of high O_2 mixtures (50-60% O_2) for 5-10 minutes does not seem to influence the course of the recovery period. These observations seem to indicate that a diminished O_2-supply for relatively short periods of time produces changes in the nervous mechanism involved in hearing which are only slowly reversible. Furthermore, they indicate that O_2-lack, CO_2-excess, and CO_2-lack influences hearing in the same fashion, although the effect of O_2-lack is most severe. Another interesting phenomenon was observed in all 3 groups of experiments. The readmission of air leads in some cases to a considerable temporary improvement in hearing. Our observations agree with those of Schubert,[2] who found that after O_2-lack upon readmission of air an improved visual discrimination is observed, as well as motor hyperexcitability. But in our experiments it is shown that such a temporary supernormal phase is not a specific reaction to O_2-lack but also occurs after CO_2-inhalation and voluntary hyperpnea.

7551 P

Influence of Variations of O_2 and CO_2 Tension in Inspired Air Upon After-Images.

ERNST GELLHORN, IRWIN SPIESMAN AND LESTER F. M. STORM.

From the Department of Physiology, College of Medicine, University of Illinois.

It was our objective to study the influence of variations of O_2 and CO_2 tension upon a simple and quantitatively measurable visual process in man. We chose the latent period of a negative after-image. The experimental subject fixated with both eyes the center of a yellow square on a grey background at a distance of 60 cm. The eyes were closed for one minute prior to each experiment.

[2] Schubert, G., *Pflüger's Arch.*, 1933, **231**, 1.

After a fixation time of 10 seconds the eyes were closed and a stop watch started. When the negative after-image appeared the watch was stopped. Control experiments showed that after a period of training the latent periods were very constant if the intervals between the individual experiments were at least 10 minutes. Fifty-five experiments were performed with 5 subjects. In the first group the influence of O_2 lack was studied by allowing the experimental subject to breathe various air-nitrogen mixtures from 7 to 30 minutes. The O_2 concentration varied between 9.2% and 16.0%. Whereas a reduction of the O_2 concentration to 13% was without influence on the latent period of negative after-images, very considerable changes occurred after breathing 9-11% O_2 for various times (7-27 minutes). The latent period was either considerably lengthened or became infinite since the after-image disappeared. In those experiments in which a negative after-image appeared, the subject noticed a decrease in its intensity. In some cases the latent period of the after-image remained lengthened even 10 minutes after the end of the breathing period. The subsequent experiments showed approximately the same latent periods as before the O_2-lack experiment.

Similar experiments were carried out in order to study the influence of CO_2. They showed that CO_2 is without effect upon the latent period of negative after-images when breathed in concentrations of 2-2.5% for as long as 26 minutes. The threshold concentration seems to be about 3-3.5%. A distinct increase in the latent period is observed in experiments with 4-7% CO_2 which was breathed for a period of 4-20 minutes. The effects were reversible.

A final series of experiments was carried out in order to study the influence of a reduction in the CO_2 tension upon sensory function. The subjects breathed with the rhythm of a metronome (between 64 and 90 per minute) for periods of 3-6 minutes. Immediately afterwards the after-images were investigated and showed regularly an increase in latent period.

The observations show conclusively that O_2 lack, CO_2 excess, and CO_2 lack have the same end effect on the sensory mechanism involved in the production of after-images. An interpretation of our experimental data in regard to the site of action will be postponed until more material on other sensory functions is available but it may be said that the effects are not due to circulatory disturbances. The blood pressure was either unchanged or varied only a few millimeters, whereas much greater alterations in blood pressure induced by physical exercise were without influence upon the latent period of after-images.

Pacific Coast Section.

University of California, June 21, 1934.

7552 C

Carbohydrate-Fibrinolytic Linkage in Streptococcus hemolyticus.*

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The discovery by Lancefield[1] of a human-diagnostic "carbohydrate" fraction in certain pathogenic strains of *Streptococcus hemolyticus,* and Tillett and Garner's[2] demonstration of a specific fibrinolytic function in similar streptococci, suggests a possible genetic linkage between these 2 hereditary (or acquired) specific bacterial characters. To test this possibility, 189 strains of hemolytic streptococci previously titrated for their fibrinolytic function[3] have

TABLE I.

Lancefield Human-diagnostic Carbohydrate Titer of Fibrinolytic Streptococci. The cultures are grouped with reference to their quantitative fibrinolytic function. Specific carbohydrate titrations were made by the Lancefield technic: (a) Ring test, 30 minutes, 37.5° C., (b) Dilution test, 18 hours, ice chest.

Specific fibrinolytic titer		Human-diagnostic carbohydrate titer	
No. of strains tested	Lytic titer	Ring test (30 min.)	Dilution test (18 hr.)
A. *S. hemolyticus* of clinical origin			
1	++++	++++	++++
17	++++	+++	++++
12	+++	+++	++++
15	++	+++	++++
2	+	++	+++
100	0	0	+±
4	0	0	0
B. *S. hemolyticus* of veterinary origin			
3	+	0	±
38	0	0	0
C. *S. viridans* of clinical origin			
33	0	0	0

* Work supported in part by CWA, in part by the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

[1] Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571.

[2] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

[3] Madison, R. R., Proc. Soc. Exp. Biol. and Med., 1934, **31**, 1018.

been retitrated for their specific carbohydrate fraction by the Lancefield technic. Control tests were run with 2 human (C 203 and K 96) and 2 veterinary (P 454 and K 158 E) strains kindly furnished by Dr. Lancefield. The results of these titrations are summarized in Table I.

Within the limits of the experimental error, there is an exact correlation between the Tillett-Garner specific fibrinolytic titer of *S. hemolyticus* and their Lancefield human-diagnostic carbohydrate titer by the ring test.

7553 C

Immunological Types of Fibrinolytic Streptococci.*

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In order to test the possibility of there being more than one immunological type of fibrinolytic streptococci, 40 local strains of *Streptococcus hemolyticus* were titrated against various specimens of normal and immune human plasma-clot. To make these titrations parallel Tillett-Garner tests[1] were run with 1:1, 1:2, 1:4, 1:8, and 1:16 dilutions of 24-hour broth filtrates of the strains in question. The maximum dilution giving distinct fibrinolysis by the end of 24 hours was recorded as approximate lytic titer for a given blood sample. A preliminary series of duplicate tests showed that the experimental error in such titrations is not greater than one dilution either way from the recorded titer.

Data from 2 typical titrations are recorded in Table I. The 2 immune plasmas here recorded were drawn from convalescent cases, one of 90 days', the other of 12 months' duration.

Adopting the plasma-clot Van. as the arbitrary standard, the table shows a normal range of fibrinolytic susceptibility of human blood varying from 4 times to 0.6 of the arbitrary standard.

There is apparently but one fibrinolytic type among the 40 streptococcus strains tested. The immune plasmas are consistently re-

* Work supported in part by the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

[1] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485. VanDeventer, J. K., and Reich, T., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 821. Madison, R. R., *Ibid.*, 1934, **31**, 1018.

TABLE I.

Fibrinolytic titration of *S. hemolyticus.*

The table records the highest dilution of the 24-hour Chamberland filtrate giving distinct fibrinolysis.

Strain No.	Fibrinolytic titer with normal plasma-clots			Immune plasma-clots	
	Rach.	Rei.	Van.	Con.	Sim.
135	16	8	2	0	0
10	8	4	4	1	0
28	8	4	4	0	0
95	8	4	1	0	0
93	4	2	1	0	0
46	4	4	0	0	0
168	1	0	0	0	0
175	1	0	0	0	0
41	0	0	0	0	0
Average titer	5.5	3	1.3	0.1	0
Relative susceptibility	4	2.3	(1)	0.1	0

Strain No.	Fibrinolytic titer with normal plasma-clots				Immune plasma-clot
	Muel.	Van.	Mad.	Till.	Sim.
30	16*	16*	16*	16	0
3	16*	16*	16	4	0
91	8	2	1	1	0
22	8	2	1	0	0
4	4	4	1	1	0
61	4	2	1	1	0
94	1	0	0	0	0
15	0	0	0	0	0
Average titer	7	5	4.5	3	0
Relative susceptibility	1.4	(1)	0.9	0.6	0

*Dilutions above 1:16 not tested.

sistant to all strains. Within the limits of the experimental error (one dilution, plus or minus) all normal plasmas are consistently susceptible.

7554 P

Intranuclear Inclusions in Brain of Chick Embryo after Inoculation of Egg with Virus of Equine Encephalomyelitis.

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From the George Williams Hooper Foundation, University of California, San Francisco, Cal.

Recently intranuclear inclusions have been described by Hurst[1] in the nerve cells of animals suffering from equine encephalomyelitis.

[1] Hurst, E. W., *J. Exp. Med.*, 1934, **59**, 529.

These bear a close resemblance to those occurring in Borna disease and in poliomyelitis. It becomes of interest to report the finding of inclusions in the brain of the chick embryo after inoculation of the developing egg with the virus of equine encephalomyelitis.

The procedure of inoculation of the developing chicks and membranes has been adequately described by Higbie and Howitt[2] as well as a study of the propagation and neutralization of the virus *in vivo*. Material obtained from this experimental work was fixed either in Zenker's fluid or in saturated corrosive sublimate containing 5% glacial acetic acid. The 2 to 5 micra sections of the membranes and embryos were stained with Giemsa or phloxine-methylene blue. Two series of sectioned embryos and membranes representing 3-hour intervals over a period of 24 hours were available for study. The New Jersey strain of virus and the California strain being represented in each of one series.

A noticeable edema of the chorio-allantoic membrane appeared in 3 to 6 hours following inoculation of the 10 to 12-day incubated egg. Normal horse serum was found to provoke a similar reaction but to less degree while physiological saline failed to produce an edema of the membranes. The swelling and gelatinous appearance of the membranes onto which the virus had previously been dropped rapidly increased and by 18 to 21 hours there was a collapse of many of the vessels and slight patchy streaking of the membranes. No definitely circumscribed areas of infection such as occur when herpes simplex is cultivated on the chorio-allantoic membrane (Dawson[3]) are seen.

Microscopically the membranes first show a thickening of the mesothelial layer and this subsequently becomes more prominent. There is no increase in the numbers of cells in this layer but the intercellular spaces are greater. In 9 hours there is a tendency towards ectodermal proliferation and slight inflammatory reaction. This process is more or less generalized, different areas revealing these reactions in varying degrees. In later stages a necrosis of the ectodermal layer with breaking up of the nuclear chromatin into irregular shaped blocks, ballooning of nuclei and necrosis of cytoplasm results. No intranuclear inclusion bodies are to be found in the cells of the membranes.

The brain of the embryonic chick is markedly softened, edematous and congested with prolonged cultivation of the virus. The New Jersey strain of the virus caused these changes in a shorter period of

[2] Higbie, E., and Howitt, B. F., in manuscript.

[3] Dawson, J. R., *Am. J. Path.*, 1933, **9**, 1.

time than did the California strain. Inclusion bodies in the embryonic nerve cells were more numerous in the various regions of the brain with the former virus. No definite regions of the brain contained greater numbers of inclusions except larger nerve cells were more disposed to their formation. No intranuclear inclusion bodies were found in the embryonic nerve cells of the spinal cord. They made their first appearance after the ninth hour and were abundant in 18 to 24 hours. It is of interest in this regard to note that Higbie and Howitt found the virus to be present in the brain of the chick embryo in 9 hours and thereafter.

The inclusions are rounded acidophilic masses usually located nearer the periphery of the nucleus. They are variable in size, ranging from tiny bodies surrounded by an halo and occupying only a small part of the nucleus, to those which appear to make up one-fourth to one-third of the nuclear volume. Usually there is one inclusion body to a nucleus but more than one is by no means rare; they seldom exceed 2 in a single nucleus. There are in addition to inclusion bodies certain necrotic nerve cells which stain deeply with phloxine and bear a resemblance to nerve cells found in acute poliomyelitis. The nucleus of such a cell is shrunken, the marginated chromatin no longer stains blue and intranuclear inclusions may be present.

To determine if similar bodies appeared after inoculation of certain other neurotropic viruses into the 10-day incubated egg Borna disease and poliomyelitis viruses were utilized. Embryos and membranes were taken from a series of eggs every 6 hours over a period of 12-72 hours following inoculation of the eggs with the virus. In no instance were intranuclear inclusion bodies of the type found in the nerve cells of embryos infected with equine encephalomyelitis apparent in the brains of the embryos infected with Borna disease or poliomyelitis. The inclusions described by Hurst are similar in appearance to those in the embryonic nerve cells of the developing chick. The most obvious difference between the two is the relatively larger size of the inclusions in the embryonic nerve cells.

7555 P

Inhibition of Yeast Growth by 2-4 Dinitrophenol.*

A. W. MARTIN AND J. FIELD II.

From the Department of Physiology, Stanford University.

The undissociated acid form of 2-4 dinitrophenol (alpha-DNP) has been shown by Field, Martin and Field[1, 2] to stimulate both the rate of oxygen consumption and of fermentation by yeast.

Is this heightened metabolic level reflected in an increase in the growth rate? To test this we have grown yeast in the L-shaped rocker tubes designed by Fraser,[3] rocking 42 times per minute through an angle of 50°, in a water bath with temperature maintained at 25° C. The medium used was 10% yeast autolysate[4] in 0.2 M phosphate buffer at pH 6.8. The solution contained 2% to 5% glucose. Adequate precautions were taken to prevent contamination during inoculation and sampling, and our pure strain[1, 2] of *Saccharomyces cerevisiae,* race F, was used. Under these conditions there was little or no change in pH over a period of 4 days. Use of such yeast, tubes and media gave good checks (within 5%) in the control tubes.

The concentration of the sodium salt of alpha-DNP which gave maximum stimulation of respiration at pH 6.8 was 400 mg. per liter or 1.785×10^{-3} molar.[1,2] This gives a concentration of 2.87×10^{-6} molar for the free acid form. Instead of a stimulation of growth, a marked inhibition occurred with this dosage, there was an increase in duration of the lag and log phase (nomenclature of Buchanan[5]) and a decrease in the total population attained, as shown in Fig. 1. All of the free acid concentrations tried by us, ranging from 7.18×10^{-8} to 7.18×10^{-6} molar, had a more or less inhibitory effect on growth.

Hopkins tube readings agreed with the cell counts in showing that at no period did the tubes containing alpha-DNP attain either

* Supported in part by a grant from the Rockefeller Fluid Research Fund of the Stanford University School of Medicine.

[1] Field, J. II, Martin, A. W., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 56.

[2] Field, J. II, Martin, A. W., and Field, S. M., *J. Cell. and Comp. Physiol.*, 1934, **4**, 405.

[3] Fraser, C. G., *J. Physical Chem.*, 1921, **25**, 1.

[4] Orla-Jensen, S., "The Lactic Acid Bacteria," Copenhagen, 1919.

[5] Buchanan, R. E., *J. Infect. Dis.*, 1918, **23**, 109.

the number of cells or the quantity of protoplasm of the control tubes.

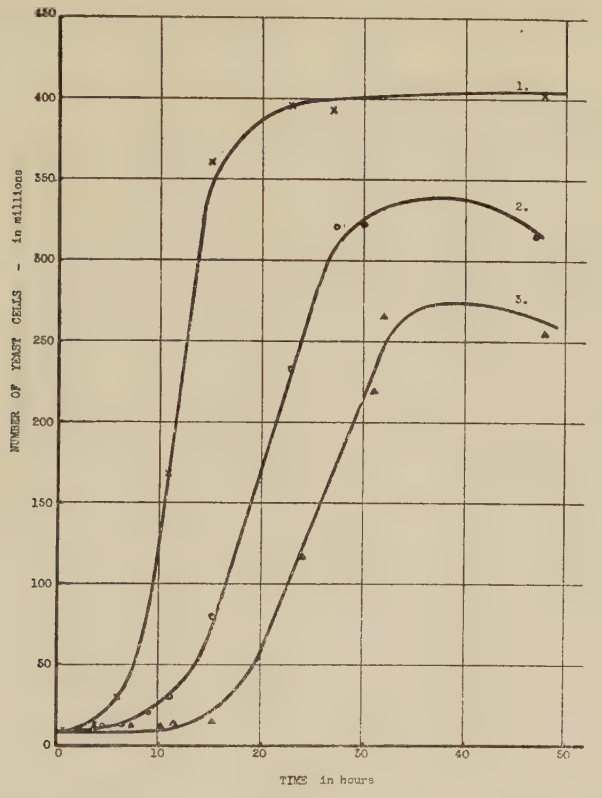

FIG. 1.

Comparison of lag and log phases of yeast growth from identical seedings. All tubes contain 5% glucose and 10% yeast autolysate made up in 0.2 M phosphate buffer, pH 6.8. Concentration of free acid alpha-DNP is 0 in tube 1, 1.795×10^{-6} molar in 2, 2.87×10^{-6} molar in 3.

Testing the yeast grown in this manner in the Warburg respirometers we found that the alpha-DNP optimum for respiration has been shifted in each case to a lower value. This can probably be explained by residual alpha-DNP in the cells in spite of the repeated washings.

A striking effect, perhaps due to acclimatization or selection of yeast cells, or both (compare Fulmer[6]) is the greatly increased respiratory rate per 10^8 cells observed with yeast grown in tubes containing alpha-DNP. This may reach 300% of the control level.

Further work is in progress.

[6] Fulmer, E. I., *J. Physical Chem.*, 1921, **25**, 455.

7556 P

Susceptibility of Non-Immune, Hyperimmunized Horses and Goats to Eastern, Western and Argentine Virus of Equine Encephalomyelitis.

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Through the studies of TenBroeck and Merrill[1] and Giltner and Shahan[2] it is known that the Eastern viruses of equine encephalitis isolated during the epidemic of 1933 are serologically distinct from those responsible for the disease in California, Nevada, Colorado, Utah and South Dakota. Rosenbusch[3] studied a similar if not identical malady in Argentine. He found the South American virus immunologically identical with the California virus. A comparative study of the 3 viruses, on horses, suggested itself. The senior author is familiar with the clinical and pathological findings observed in horses during the 1912 epidemic in Delaware and during the outbreaks in California (1930 to 1933), Colorado and Texas. The results of these studies may be summarized as follows:

(1) Two Eastern viruses (Delaware isolated from a brain sent to California in glycerine, New Jersey courteously furnished by Dr. Carl TenBroeck) infected horses when injected intracerebrally, intravenously (filtrates) and intracutaneously. In one series 1 cc. of a 20% suspension of guinea pig passage virus fatally infected 4 of 14 horses injected intracutaneously on the neck (distribution of cervical nerves). The clinical picture (fever, stupor and motor disturbances) differed in no way from that recorded in the many horses infected with the Western virus, although the course of the disease was greatly accelerated. Several animals died in from 62 to 163 hours after the administration of the virus or they were unable to rise between the 80th and 96th hour and were consequently sacrificed. Irrespective of the mode of infection, the virus was demonstrable in the blood serum from the 12th to 65th hours. The spinal fluid was invariably increased and turbid with a cell count of from 600 to 25,000 cells (5 to 20% granulocytes), and a positive Rivolta test and occasional web formation. The gross

[1] TenBroeck and Merrill, PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 217.

[2] Giltner and Shahan, *Science*, 1933, **78**, 587.

[3] Rosenbusch, *Anales d. l. Sociedad Rural Argentina*, 1934, and personal communication to senior author.

anatomical lesions were slight but the histologic examination revealed very extensive and pronounced degenerations with inflammatory alterations in the central and peripheral nervous system.

(2) A virus obtained from a fatal infection in Utah and the Argentine virus infect by the intracerebral route only. Filtrates injected intravenously or suspensions administered intracutaneously fail to incite the disease.

(3) Thirty horses injected subcutaneously with 5 cc. of a 20% suspension of California guinea pig passage virus had slight febrile reactions. Subsequently they tolerated large doses of Western virus.

(4) Of 3 horses previously hyperimmunized for 2 years with California, Nevada and South Dakota virus and injected intracerebrally with Delaware and New Jersey virus, 2 reacted severely and were sacrificed for humane reasons. One horse had a sharp febrile reaction and transitory symptoms of encephalitis but recovered. It had been previously recognized that the intracerebral mode of infection is an exceedingly drastic method to determine the immunity of horses against encephalitis (Meyer, Haring and Howitt, Records and Vawter), and doubtless not well suited for cross-immunity tests.

(5) Twelve horses highly hyperimmunized for several months with Western viruses yielding a serum with antiviral substances injected intracutaneously with 1 or 2 cc. and subsequently with 4 cc. of a 20% suspension of Eastern virus survived. Several of the animals had slight transitory febrile reactions (39 to 40° C.). Four control horses injected simultaneously by the same route developed typical encephalomyelitis, and either succumbed to the disease or were sacrificed.

(6) Two horses hyperimmune to the Western virus and injected intracerebrally with Argentine virus showed no signs of illness. The control horse developed encephalitis and finally succumbed. During the febrile reaction the virus was demonstrated for from 12 to 60 hours in the blood serum. Simultaneously, horses hyperimmune to the California and Nevada virus were tested intracerebrally with the Utah virus. They failed to react.

(7) One horse, which had survived an intracerebral injection of Argentine virus, tolerated the intracutaneous injection of the Eastern virus (4 cc.).

(8) Two horses injected at weekly intervals with Eastern virus (1, 2 and 5 cc. of a 20% virus suspension) failed to react to an intracerebral injection (5 cc.) of a potent California virus, which fatally infected (with 2 cc.) a normal animal.

(9) The Eastern, Western and Argentine viruses injected intracerebrally may produce in goats a transitory or a rapidly fatal encephalitis.

These observations indicate a very close relationship between the Western and Argentine virus not only with respect to infectivity but also with regard to cross protection. By contrast the Eastern virus of 1933 exhibits a greater virulence and thus may break the immunity established against the Western virus. On the other hand, the Eastern virus apparently protects against the Western virus. Further, these experiments lend considerable support to the conception of an insect transmission of the encephalitis virus as demonstrated by Kelser. The Eastern virus infects readily by the cutaneous route and the infective agent circulates for many hours in the blood of the horse.

7557 P

Propagation of Virus of Equine Encephalomyelitis after Intranasal Instillation in the Guinea Pig.

B. F. HOWITT. (Introduced by K. F. Meyer.)

From the George Williams Hooper Foundation, University of California, San Francisco, California.

In an attempt to determine whether or not the virus of equine encephalomyelitis reported by Meyer, Haring and Howitt[1] spreads by way of an initial blood stream invasion and secondary penetration of the meningo-choroid plexus or by axonal propagation as in poliomyelitis,[2–5] the distribution of the infective agent in the tissues of guinea pigs following the intranasal instillation of the virus was studied.

Several series of small guinea pigs were each given 2 cc. of a 20% saline suspension of California virus dropped into the nares. Three animals were killed by bleeding from the heart at each of the different periods of time as shown in Table I. No blood was removed at the twelfth hour, however. The tissues were removed

[1] Meyer, K. F., Haring, C. M., and Howitt, B., *Science*, 1931, **74**, 227.

[2] Faber, H. K., and Gebhardt, L. P., *J. Exp. Med.*, 1933, **57**, 933.

[3] Flexner, S., *Science*, 1933, **77**, 413.

[4] Brodie, M., and Elvidge, A. R., *Science*, 1934, **79**, 235.

[5] Schulze, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 728.

TABLE I.

Chronological Appearance of the Virus of Equine Encephalomyelitis in the following tissues:*

Hrs.	Serum	Filtered Nasal Mucosa	Cervical Glands	Olfactory Bulbs	Maxillary and Sub-maxillary Glands	Pons and Medulla	Cerebellum	Corpora Quadrigemina	Thalamus	Cerebral Hemispheres	Hind Brain	Midbrain	Anterior end of Forebrain
4	0	1st Series 0 2nd Series +	0	0	0							0	
8	+	+	0	0	0						0	0	0
12		+	0	0	0							0	
15	+	0	+	0	0						0	0	0
18	+	+	+	+	0						0	0	0
19	+	+	+	0	+						0	0	0
20	+	+	+	+	0						+	0	0
21	+	+	+	+	+	+	+	0	0	+			0
22	+	+	+	+	+	+	+	+	+	+			+
23	+	+	+	+	0						+	0	+
24	+	+	+	+	+	+	+	+	0	0			1st series 0 2nd series slt. +
36	+	0	+	+	+						+	+	+
48	+	+	+	+	+						+	+	+
96	+		+	+	+	+	+	+	+	+	+	+	+
120	0	+	+	+	0						+	+	+

* + = virus present; 0 = no virus recovered.

aseptically, pooled from the 3 animals, ground with saline and injected intracranially into guinea pigs. The nasal mucosa was extracted in saline and passed through a Seitz filter before inoculation. The serum was given both into the brain and subcutaneously. Several guinea pigs were allowed to live until prostration as controls on the viability of the virus. They all succumbed to the disease.

The results as given in Table I show an immediate and earlier invasion of the blood stream for this experiment than that previously reported[6] for guinea pigs. The virus was constantly present in the serum after the eighth hour and was almost always recovered from the nasal mucosa. Its constant presence in this tissue, however, may have been due to the amount of blood necessarily obtained from this region. It was then recovered from the cervical and salivary glands, the olfactory bulbs and subsequently from the cerebral nerve tissues beginning with the hind brain. After the 36th hour, coincident with the rise in temperature, the virus was constantly present in all of the tissues examined, except for the disappearance from the blood after defervescence and the subsequent prostration of the animal.

To further corroborate the evidence just given of the primary invasion of the blood stream rather than the nerve tissues, an experiment similar to that described by Brodie[4] and by Schulze and Gebhardt[5] for poliomyelitis in monkeys was performed on guinea pigs. The olfactory bulbs were removed surgically from 4 guinea pigs under anesthesia. After one week they, together with 4 normal animals were given 2 cc. of virus intranasally. All 8 guinea pigs succumbed to the disease with typical symptoms. To show that the virus had not been absorbed through the digestive tract, 2 more animals were fed 2 cc. each of the same virus by catheter into the stomach. Neither of them developed the disease.

From the evidence obtained it would seem that the virus of equine encephalomyelitis when given intranasally, gains entrance primarily into the blood stream, presumably through the vascular nasal mucosa and that there is a systemic septicemic invasion before localization in the nerve tissue. A more detailed report of this study will be given later.

[6] Howitt, B. F., *J. Infect. Dis.*, 1932, **51**, 493.

7558 P

Experimental Studies on Trypanosoma cruzi in California.

FAE DONAT WOOD. (Introduced by C. A. Kofoid.)

From the Zoology Department and Hooper Foundation, University of California.

The reduviid, *Triatoma protracta* Uhler, was reported to be a carrier of *Trypanosoma cruzi* Chagas, the haematozoon causing American human trypanosomiasis.[1] Examinations have revealed that the feces of 40 out of 73, or 54%, of a group of *Triatoma* from San Diego County were infected with *T. cruzi*. *Triatoma* from the vicinities of Berkeley and Los Angeles have not shown trypanosomes in their digestive tracts.

The San Diego wood rat has been incriminated as a reservoir host of *T. cruzi*. Of 43 rats examined only one was infected. This was a light infection and was detected by examination of centrifuged, citrated heart blood. The trypanosome infecting this rat was identical in morphology and behavior with that found in animals infected experimentally from *Triatoma* feces, and also with a known strain of *T. cruzi* from Professor Brumpt's Paris laboratory.

The southern parasitic mouse, the San Diego desert mouse, and the Virginia opossum, all associated with San Diego wood rats in nature, are more susceptible to the infection in the laboratory than the rats themselves, so it is possible that they, too, may be natural carriers of this trypanosome.

Portola wood rats, from Berkeley, harbor a trypanosome of the "lewisi" type which should not be confused with *T. cruzi*.

One hundred thirty-four animals, including 16 species, have been inoculated with the California strain of *T. cruzi*. The following list gives the species and number of animals inoculated, the number in parenthesis indicating how many animals became infected: 53 (30) albino Norway rats, 23 (7) albino mice, 2 (1) puppies, 2 (1) Virginia opossums, 4 (3) rhesus monkeys, 12 (7) San Diego wood rats, 3 (1) Portola wood rats, 5 species of white-footed mice [4 (4) parasitic, 9 (8) southern parasitic, 3 (3) San Diego desert, 5 (1) Gambel, 3 (1) Gilbert], 2 (0) rabbits, 6 (0) guinea pigs, 3 (0) kittens, and 1 (0) desert antelope ground squirrel.

Infection has been produced by the following methods of inoculation: infective *Triatoma* feces intraperitoneally, subcutaneously, intramuscularly, in the eyes, mouth, or on scarified skin; citrated

[1] Kofoid, C. A., and Donat, F., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 489.

blood intraperitoneally, subcutaneously, or intramuscularly; and culture forms intraperitoneally.

Groups of leishmaniform bodies have been found in bone marrow and cardiac and voluntary muscles of infected animals. Lesions composed of infiltrating lymphocytes, monocytes, and plasma cells were seen in cardiac and voluntary muscles, cerebrum, and meninges. Some cases have shown fatty degeneration of the liver.

The parasites, both in the blood and in the tissues, have been very scarce in most cases. The only animal to show any symptoms, namely retarded growth and the temporary paralysis of the hind legs, was the southern parasitic mouse. In no case was the disease fatal.

Attempts were made to intensify the infection by lowering the host's resistance by splenectomy, by injection of testicle extract, and by keeping the animals at a higher temperature.

Previously[1] splenectomy seemed to stimulate the appearance of *T. cruzi* in latent infections. Further experiments with a larger number of animals indicate that splenectomy has no real effect. Eight (57%) out of 14 splenectomized, and 22 (56%) out of 39 non-splenectomized, albino rats became infected after inoculation. The difference of 1% can hardly be considered significant.

Duran-Reynals[2] reported that injection of testicle extract increased the invasiveness of a neurovirus in rabbits. Experiments indicate that testicle extract has no such stimulating effect upon *T. cruzi*.

Six albino mice placed in an incubator at 34 to 36° C. did not take heavier infections than controls kept at room temperature.

Successive passages through different host species (puppy, albino rat, San Diego wood rat, albino rat), covering a period of 103 days indicated a stimulating effect upon the trypanosomes in that the incubation period progressively decreased (35, 26, 22, 20 days). In 2 control experiments in which the trypanosome was passed through animals of the same species, *i .e.,* young albino rats or mice, the parasites failed to appear after the eighth and fourth passages, respectively.

T. cruzi has been successfully cultured and subcultured on semi-solid blood-agar. Material from the original cultures and from the first subcultures has produced typical infections in white-footed mice.

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[2] Duran-Reynals, F., *J. Exp. Med.*, 1929, **50**, 327.

7559 P

Inhibition by Glucose of Methemoglobin Formation.

MATILDA MOLDENHAUER BROOKS.

From the Department of Zoology, University of California, Berkeley.

The purpose of these experiments is to show that injection of glucose prevents the formation of methemoglobin in animals, or if methemoglobin is already present, it can by the same means be reduced to hemoglobin which is then readily oxygenated to oxyhemoglobin.

Methemoglobin was produced experimentally in rabbits by injections of 0.15 gm. of $NaNO_2$ for every kg. of body weight. This dose converted 15% of the blood-hemoglobin into methemoglobin in a few minutes. This is less than the theoretical effect predicted by Wendel[1] for this dose, owing no doubt to the constant presence of glucose normally in the blood stream. Glucose was used in amounts of 1 cc. to 2 cc. of a 1% solution for every kg. body weight. All solutions were made up fresh with 0.9% NaCl and injected intravenously into one ear of the rabbit; blood samples were taken from the other ear at intervals thereafter.

The spectrophotometric method was used for determining the proportions of methemoglobin in blood. This method is sensitive to less than 2%. The ratio, R, of the extinction coefficient at $\lambda = 540$ mμ to that at $\lambda = 560$ mμ, as found by Ray, Blair and Thomas[2] indicates the per cent of methemoglobin present. All blood samples were diluted to 1% with 0.4% NH_4OH. No difference in readings was noted when water was used as a diluent. The thickness of the layer measured was 1 cm. Five animals were used in each group. The probable error of the readings was a fraction of 1%. The method is also valuable because determinations can be made so quickly that chemical changes following collection are minimized.

Results *in vivo*. Methemoglobin was produced in the rabbit as indicated above, the R value found being 1.56 which indicates 15% conversion to methemoglobin. Glucose was then injected. Five minutes later the R value was 1.66 which indicates complete reconversion of methemoglobin to oxyhemoglobin. The controls which had not received glucose still showed 15% methemoglobin present. If glucose is injected before the nitrite, no methemoglobin can be demonstrated even after several hours. Injections of saline in the

[1] Ray, G. B., Blair, H. A., and Thomas, C. I., *J. B. C.*, 1932, **98**, 63.

[2] Wendel, W. B., *J. Am. Med. Assn.*, 1933, **100**, 1054.

place of glucose have no effect on methemoglobin formation or its change to oxyhemoglobin.

Results *in vitro* with sheep blood also showed that methemoglobin formation is delayed if glucose is present, or if already formed, the addition of glucose will reconvert a part of it to oxyhemoglobin. The following experiment will illustrate this: to 4 cc. of washed r.b.c. suspended in 0.9% saline solution, there was added .05 cc. of M/20 phenylhydroxylamine, freshly prepared. R was then found to be 1.42, indicating 48% methemoglobin. This solution was divided into 2 parts: glucose was added to one part, and to the other part only saline. The glucose-containing blood now had only 18% methemoglobin present ($R = 1.53$) while that with a saline alone remained unchanged at 48% ($R = 1.42$). It has not been found possible to reduce *in vitro* all the methemoglobin to oxyhemoglobin. This was also found by Warburg, Kubowitz and Christian,[3] who state that unknown reactions take place.

Similar effects can be demonstrated using $NaNO_2$ instead of phenylhydroxylamine. If glucose be added before the methemoglobin-producing substances, the formation of methemoglobin is delayed and the sample is still reddish when the control is brown.

These results show that glucose is effective in preventing methemoglobin formation, or after formation, in reducing it to hemoglobin which can then form oxyhemoglobin. It is suggested that the presence of glucose in the blood stream is responsible for the often observed failure of various agents to produce the expected proportion of methemoglobin. It is also suggested that injections of glucose be used clinically in cases where methemoglobin is present either as a result of pathological conditions or as a result of poisoning by such methemoglobin-producing substances as aniline dyes, nitrites, etc.

7560 P

Action of Respiratory Catalysts and Inhibitors on Oxygen Consumption by Nitella.

EDWARD ROSS. (Introduced by S. C. Brooks.)

From the Department of Zoology, University of California.

The material used consisted of young, actively growing coenocytic cells of *Nitella clavata* collected from a large outdoor pool. The

[3] Warburg, O., Kubowitz, F., and Christian, W., *Biochem. Z.*, 1931, **242**, 170.

growing tips of several dozen stems were cut off and kept in a large beaker of pond water in the dark for 24 hours prior to use. These clusters of cells were from 2 to 10 mm. in diameter.

The manometric technique, originally described by Barcroft and Haldane, and subsequently employed extensively by Warburg, was used for measuring oxygen consumption. The material was immersed in pond water. Five per cent KOH was used in the inset to absorb CO_2, and the vessels containing the green plants were wrapped in an opaque black cloth to exclude light. Oxygen consumption was measured as a rate, Q_{O_2}, in cmm. per hour per gram wet weight.

The effects of NaCN and methylene blue, singly and combined, were studied, purely relative results being obtained when the agents were used separately. When methylene blue was added after the cyanide, all values were corrected for the percentage change of a control run at the same time. In every case the normal Q_{O_2} was established for the second hour of a 2-hour run. In the case of cyanide the vessels were then disconnected and 0.4 cc. of the proper NaCN concentration was added, and the Q_{O_2} of the second hour thereafter determined. When methylene blue was used alone the dye was poured into the plant environment from the side-arm after the normal Q_{O_2} had been established. When methylene blue was used in combination with NaCN, the percentage inhibition was first determined with cyanide alone, the methylene blue then added and the Q_{O_2} determined for the second hour thereafter, in conformance with the standard procedure. All inhibition and acceleration were expressed as percentage deviation from the normal as first established in each case.

The results are given in Table I, negative indicating the percentage decrease in oxygen consumption, and positive the increase.

TABLE I.

	Effects of NaCN Alone					Effects of Methylene Blue Alone			
Concentration	10^{-1}M	10^{-2}M	10^{-3}M	10^{-4}M	10^{-5}M	10^{-2}M	10^{-3}M	10^{-4}M	10^{-5}M
%	−6%	−44	−46, −51	−29	−12	+107	+74	+43	+5
deviation	−5	−33	−42, −44	−20	−8	+123	+66	+48	−1
from normal	+35		−44, −51						
Aver.	+8	−39	−46	−25	−10	+115	+70	+46	+2

Since the cells were definitely and irreversibly injured in 10^{-1} M cyanide, the absence of inhibition is probably due to injury. Injury probably also decreases inhibition by 10^{-2} M cyanide, though not to so great an extent. Normal oxygen consumption was resumed

when 10^{-3} M cyanide had been washed out. There was, therefore, no irreversible injury at this or lower concentrations. Acceleration was perfectly reversible on washing out the methylene blue taken up from 10^{-4} M solution; but after 10^{-3} M it was not possible to wash out all the dye within a reasonable time. Injury cannot be surely excluded in the case of 10^{-2} M solution of the dye.

The antagonism between cyanide and methylene blue has been tested by 3 experiments so far. In all of these 10^{-3} M NaCN was used. The results, corrected for percentage changes in the controls, are given in Table II.

TABLE II.
Effects of Methylene Blue Added after Cyanide.

Experiment	1	2	3
Effect of NaCN, 10^{-3}M	—44%	—33%	—36%
Concentration of dye added	10^{-2}M	10^{-3}M	10^{-4}M
Resultant effect (0.0%—complete recovery)	—9%	—41%	—39%

The dye appears to have accelerating power only when its concentration is greater than that of the inhibitor. With this material the apparent acceleration may have been due to injury, investigation of which is in progress.

New York Meeting.

New York Academy of Medicine, October 17, 1934.

7561 C

Further Experiments on the Effect of Testicle Extract on the Agent of Chicken Tumor I.

F. DURAN-REYNALS AND ALBERT CLAUDE. (Introduced by J. B. Murphy.)

From the Rockefeller Institute for Medical Research, New York.

Hoffman, Parker, and Walker[1] reported that rabbit testicle extract markedly enhanced the growth of Chicken Tumor I, the effect being the same whether tumor mash or cell-free filtrates of the tumor were used in the inoculations. Sturm and one of us[2] could not duplicate these findings despite the use of a variety of both testicle extracts and active tumor preparations.

Since more work was indispensable in order to elucidate the cause of the discrepancies we first resorted to the technique of progressive dilution of the active tumor material. Accordingly, water dilutions of the fresh tumor tissue, ranging from 1:6 to 1:10,000 were obtained and 0.5 cc. of each dilution was injected with its volume of filtered bull testicle extract diluted 1:2. Each chicken was inoculated intradermally in 2 or 3 areas in each side of the

TABLE I.

No. of tests	Dilution of tumor extracts	Aver. area of tumor surface after 14 days		Aver. area of tumor surface after 21 days	
		Testicle extract	H_2O	Testicle extract	H_2O
		sq. cm.	sq. cm.	sq. cm.	sq. cm.
9	1:6	6.2	4.8	12.0	8.7
8	1:60	4.0	4.1	6.1	9.1
8	1:300	1.6	1.1	4.7	3.4
3	1:600	4.5	.1	.5	.0
2	1:3,000	.05	.0	.1	.0
1	1:12,000	.0	.0	3.0	.0
1	1:30,000	.0	.0	2.0	.0
1	1:60,000	.0	.0	.0	.0

[1] Hoffman, D. C., Parker, F., and Walker, T. T., *Am. J. Path.*, 1931, **7**, 523.

[2] Sturm, E., and Duran-Reynals, *J. Exp. Med.*, 1932, **56**, 711.

breast. The customary spreading was noticed after each injection. The results are summarized in Table I.

Table I shows some enhancement by testicle extract, but far less regular and marked than in the case of viruses.

In the foregoing tests, the mixture was inoculated intradermally at points located on both sides of the median breast line. With the bird in normal position this region is more or less horizontal and occupies the lowest part of the body. It is obvious that in this case the mixture injected had little opportunity to spread. Under special conditions the spreading agent may even drain back the active material. This fact may well account for the observation that in certain cases tumors obtained from testicle extract mixtures were smaller than the controls.

In the following experiments, the material was inoculated into the skin, either in a lateral region under the wing, or in the upper part of the leg. The active material was obtained by extracting fresh chicken tumor pulp with 12 volumes of sterile distilled water. The fluid after centrifugation was used for the test and mixed before inoculation with an equal volume of fresh rat testicle extract. The latter had been prepared in the usual manner by extracting with an equal volume of water, and using the supernatant fluid from centrifugation.

As illustrated in Table II, the results were conclusive and the tumors obtained were incomparably larger than in the case of the control injection, simply diluted with an equal volume of Ringer's solution.

TABLE II.

Exp. No.	No. of tests	Amt. of mixture injected	Aver. size of main tumor surface after 18 days		Aver. area of skin with scattered nodules around the main tumor	
			Testicle extract	Ringer's solution	Testicle extract	Ringer's solution
		cc.	sq. cm.	sq. cm.	sq. cm.	sq. cm.
1	1	.5	32.8	8.6	51.1	.0
2	1	.5	12.0	8.6	36.0	.0
3	2	.8	26.7	3.7	59.0	.0
4	2	.8	19.5	3.7	40.8	.0
5	3	.6	61.2	7.0	.0	.0
6	3	.6	46.0	6.3	.0	.0
7	1	.6	32.0	5.0	.0	.0
Average surface of tumor			34.0	6.1	48.4	.0

Conclusion. In agreement with the results of Hoffman, Parker, and Walker, Chicken Tumor I agent is spread when injected together with testicle extract and the resultant lesions are markedly enhanced.

7562 P

Protective Substances in Sera of Animals Injected with Anterior Pituitary-Like Hormone of Teratoma Testis Urine.

GRAY H. TWOMBLY AND RUSSELL S. FERGUSON. (Introduced by J. Ewing.)

From the Memorial Hospital, New York City.

Collip[1] reported observations on the results of repeated inoculations of the thyreotropic hormone, in large amounts, over prolonged periods of time into white rats. He showed that, on injection of the thyreotropic principle of the pituitary, hyperplasia of the thyroid gland occurs and the metabolic rate rises sharply. However, continued injections fail to maintain this condition and the metabolic rate returns to normal in from 2 to 3 weeks and may even become subnormal. The animals fail to show any rise in metabolism on the administration of as much as 8 times the previously effective dose. The serum of animals, thus rendered refractory, will exert a protective effect against the activity of the hormone when injected into untreated animals. It does not protect, however, from the action of desiccated thyroid tissue. Anderson and Collip[2] have prepared a potent antithyreotropic serum by repeated injections into a horse. Similar "antihormones" inhibiting the action of the anterior pituitary-like hormone of pregnancy urine have been made and reported by Selye, Bachman, Thompson, and Collip.[3]

In an attempt to confirm this work and to determine whether the same thing is true of the anterior pituitary-like hormone found in the urine of patients suffering from teratoma testis, we have injected a number of rabbits over prolonged periods of time. The anterior pituitary-like hormone was obtained according to the original method of Zondek[4] by precipitation of acidified fresh urine with 5 times its volume of 95% ethyl alcohol. The precipitate was washed 3 times with ether, dried, and stored in the ice box. Due to the difficulty of obtaining large amounts of the urine, the extracts were prepared from lots of 300 to 1000 cc. These were assayed separately for their potency by the injection of watery extracts into immature female mice. Most of the urine came from 2 patients and was found to contain 5,000 to 10,000 mouse units per liter.

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[1] Collip, J. B., *J. Mount Sinai Hosp.*, 1934, **1**, 28.

[2] Anderson, Evelyn M., and Collip, J. B., *Lancet*, 1934, **1**, 784.

[3] Selye, Hans, Bachman, C., Thompson, D. L., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1113.

[4] Zondek, Bernhard, *Die Hormone des Ovariums und des Hypophysenvorderlappens*, Berlin, Julius Springer, 1931.

Three litter-mate female rabbits 13 weeks old, weighing 2 kilos each, were injected with approximately 100 mouse units of an aqueous extract of the powder containing the hormone, daily for a period of 3½ months. Two days after cessation of the injections the animals were bled and the serum separated. This serum was tested for its protective effect against the action of the original hormone in the following fashion: Infantile female mice, weighing 6 to 8 gm. were injected with approximately 4 mouse units of the aqueous solution of the hormone and 0.5 cc. of the rabbit serum, over a period of 30 hours (0.2 cc. of hormone solution and 0.1 cc. of serum given subcutaneously at 12 a. m., 4 p. m., 9 a. m., 12 a.m., and 4 p. m.). One hundred hours after the beginning of the injections the animals were killed and their ovaries inspected according to the usual technique of the Aschheim-Zondek test. Thirty-nine mice so treated failed to show any corpora lutea while 24 mice receiving the extract alone all showed large corpora lutea and open vaginal orifices. Thirteen mice were given the aqueous extract plus the serum of normal rabbits. The serum was obtained from 5 different animals. All the mice so treated showed corpora lutea, that is, the sera of these rabbits failed to show any protective effect against the action of the hormone such as was exhibited by the sera of the injected rabbits.

The injection of only 0.3 cc. of serum into each mouse also protected against the action of the hormone. Three mice injected with 0.1 cc. only, showed protection in one, no protection in one, and apparently no corpora lutea but an open vaginal orifice in the third. That is, there was sufficient protective substance in 0.3 cc. of the sera of the injected rabbits to counteract completely the action of 4 mouse units of A.P.L. from teratoma urine. The experiments were clear cut, none of the mice receiving half a cc. of immune serum showing corpora lutea and all of the controls showing luteinization.

A similar experiment was tried with the preparation of the A.P.L hormone of pregnancy urine made by E. R. Squibb and Sons, called by them "Follutein". This extract in glycerine contained 250 mouse units per cc. and was said to have 0.55 mg. of N. per cc. The serum of 3 female rabbits, weighing approximately 2 kilos each, was tested for any protective effect it might show against the action of the hormone, each mouse being given 0.5 cc. of serum and 5 mouse units of Follutein. Eleven mice so tested failed to show any evidence of protective substance. The rabbits were then injected daily with 75 mouse units (375 rat units) and the injections continued, with occasional interruptions, for a month. At the end of this

time samples of the serum all showed complete protective action in 0.5 cc. amounts against 5 mouse units of Follutein. The injections were continued for another 2 weeks, at the end of which time the rabbits showed multiple sores in the skin of the back, a phenomenon which had been previously noted in the animals injected with A.P.L from teratoma testis urine. All these animals continued to show a protective effect in their serum. A male rabbit injected for a month gave similar results. Twenty-seven mice tested with the sera after injection showed complete protection. Twelve control mice receiving Follutein alone all showed corpora lutea.

Cross protection experiments of this serum and the teratoma testis urine hormone were carried out. Three mice injected with the teratoma hormone alone showed corpora lutea while 9 receiving 4 mouse units of the hormone plus 0.5 cc. of serum immunized against the pregnancy hormone each showed no effect in the ovaries. Nine mice receiving four mouse units of "Follutein" plus 0.5 cc. of serum from the rabbits immunized against the teratoma hormone showed no evidence of luteinization while three control mice injected with "Follutein" alone showed corpora lutea.

7563 P

Successive Transmission of Virus of Lymphogranuloma Inguinale Through White Mice.

ARTHUR W. GRACE AND FLORENCE H. SUSKIND.
(Introduced by Paul Reznikoff.)

From the New York Hospital and Department of Medicine, Cornell University Medical College, New York.

As part of a study of the properties of the virus of lymphogranuloma inguinale white mice were inoculated intracerebrally with bacteriologcally sterile pus aspirated from an inguinal bubo and glandular material obtained from a case of lymphogranuloma inguinale. The pus and glandular material were diluted 1 in 5 with sterile distilled water and inoculated in 0.03 cc. quantities into each of 6 mice. The object of this section of the work was to ascertain whether or not the virus could be transmitted indefinitely in that manner. All of the inoculated animals died within an average of 11 days. The brain of one of these animals dying from lymphogranuloma inguinale was emulsified in 1 in 2.5 dilution of distilled water and inoculated intracerebrally in 0.03 cc. quantities into another batch of 6 white mice. All of these mice died within an

average of 7.0 days. A similar procedure caused the death of the next four generations of mice in an average of 5.4 days and by the time the eleventh generation was reached, the animals were expiring in four days. Similarly, there was an increased mortality rate with successive passage, 90 to 100% of all the inoculated animals succumbing in the seventh to the eleventh generations. The shortening of the period taken for the mice to die involved such frequent subpassage that the strength of the inocula of subsequent generations was reduced to a 1 in 5 dilution. The effect of the increased dilution was to make the average time taken for the mice to die longer and to decrease the mortality rate. Nevertheless, there continued to be an increase in the virulence of the virus so that by the 26th and 27th generations, when a 1 in 2.5 dilution was again employed, 100% of all the inoculated animals died in an average of 2.6 days. At the time of writing (35th generation) 80 to 100% of mice die in an average of 6.7 days as a result of an intracerebral inoculation of a 1 in 5 suspension of lymphogranulomatous mouse brain. Physiological saline, Tyrode's solution, ascitic fluid or infusion broth of pH 8.0 are diluents equally as good as sterile distilled water.

A control series of inoculations in which normal mouse brain was used instead of lymphogranulomatous mouse brain, did not produce any effect in mice.

Evidence of the presence of the virus of lymphogranuloma inguinale in the brains of the dead mice was furnished by the production of highly potent Frei antigens from these brains; the method of preparation of the mouse brain antigen was identical with that used for the preparation of Frei antigens from human lymphogranulomatous pus. The potency of the antigen appears to increase with successive passage of the virus. Normal mouse brains prepared and tested as Frei antigens do not produce any appreciable reactions.

Histological examination of the brains of the dead mice indicates that the virus produces a meningitis in which the predominant cell is of the lymphocyte type; polymorphonuclear leucocytes are comparatively sparse.

Conclusion. One strain of the virus of lymphogranuloma inguinale, upon intracerebral inoculation into white mice, has quickly developed a fixed virulence for these animals and appears to be capable of transmission indefinitely in this manner.

Frei antigens prepared from lymphogranulomatous mouse brains are specific and highly potent; the antigenic strength increases with successive mouse passage.

7564 P

Renal Excretion of Inulin, Creatinine and Xylose in Normal Dogs.

A. N. RICHARDS, B. B. WESTFALL AND PHYLLIS A. BOTT.

From the Laboratory of Pharmacology, University of Pennsylvania.

During the course of studies of the properties of the renal tubule of amphibia we had occasion to search for a substance which, introduced into the tubule, would not be expected to be absorbed from it into the blood, either actively or by diffusion. Among those tried was the polysaccharide inulin, chosen because of its high molecular weight and because it is not hydrolyzed by enzymes or tissues of vertebrates. After having found that inulin is much less rapidly diffusible than either creatinine or glucose; that it is filterable through collodion membranes which are impermeable to protein and through the glomerular membranes of amphibia; and also that it is not excreted by the aglomerular kidney (toadfish) after intravenous injection, it seemed desirable to study its rate of excretion in mammals. Given intravenously to dogs or rabbits it is excreted in the urine rapidly, and, insofar as a few experiments show, completely. Concentration ratios, U/P, as high as 150 have been observed. It became obvious that some of the considerations developed by Jolliffe, Shannon and Smith[1] upon which they based their advocacy of plasma clearance of xylose in preference to that of creatinine as a true measure of the volume of glomerular filtration could equally well be made the basis of similar advocacy of inulin clearance for the same purpose; with this advantage, that the low diffusibility of inulin, much lower than that of creatinine, of xylose and of the other 2 non-metabolized sugars tested by them (sucrose, raffinose) could be expected to minimize a difference between plasma clearance and rate of glomerular filtration, provided such a difference exists and is the result of back diffusion of the substance studied. Accordingly, a series of preliminary experiments has been made, admittedly not perfect, in which the simultaneous plasma clearances of inulin and creatinine have been determined in unanesthetized female dogs. The results, which seem sufficiently convincing to put on record, indicate that the plasma clearance of inulin given intravenously is slightly higher than, but of the same order as that of creatinine.

[1] Jolliffe, N., Shannon, J. A., and Smith, Homer W., *Am. J. Physiol.*, 1932, **100**, 301; **102**, 534.

A year later a similar series was made in which simultaneous clearances of inulin and xylose were determined. The results indicate that inulin clearance is significantly higher than that of xylose.

Inulin was given intravenously: 1 to 4 gm. before beginning the experiment; slow continuous infusion during it. Creatinine was given intraperitoneally, xylose by mouth.

Urine collection periods varied from 12 to 52 minutes; average, 27. Blood from the jugular vein was taken one minute before the beginning and one minute before the end of each period. Urine was collected by catheter.

Inulin and xylose in plasma and urine were determined by the Shaffer-Somogyi method[2] applied to the properly diluted fluids (a) before hydrolysis, (glucose + xylose); (b) before hydrolysis, after fermentation with washed yeast (xylose); (c) after hydrolysis with 0.1 N HCl (glucose + xylose + inulin). Urines were diluted to make the reducing powers approximately the same as those of the corresponding plasmas.

Creatinine was determined by Folin's method as used by Holten and Rehberg.[3]

Twenty-three comparisons of inulin and creatinine clearances were obtained in 10 experiments on 5 normal dogs in June and July, 1933; 11 more in 5 experiments on 4 dogs in July, 1934.

Twenty-five comparisons of inulin and xylose clearances were made in 11 experiments on 4 normal dogs in May, June and July, 1934. In any single experiment clearance of only 2 of the 3 substances was measured.

Average plasma concentrations of inulin ranged from 60 to 346; of creatinine, from 3.3 to 26.0; of xylose, from 30 to 233 mg. %. Rates of urine excretion varied from 0.44 to 5.8 cc. per min.

The results, given as clearance ratios, were as follows:

A. *Inulin clearance/Creatinine clearance:* 0.64, 0.64, 0.73, 0.84, 0.85, 0.87, 0.92, 0.92, 0.96, 0.97, 1.00, 1.00, 1.01, 1.02, 1.03, 1.03, 1.05, 1.05, 1.08, 1.11, 1.11, 1.14, 1.14, 1.14, 1.15, 1.16, 1.18, 1.20, 1.20, 1.21, 1.36, 1.38, 1.46, 1.53. Mean, 1.06.

B. *Inulin clearance/Xylose clearance:* 0.85, 0.89, 0.93, 1.03, 1.08, 1.09, 1.11, 1.16, 1.16, 1.16, 1.23, 1.25, 1.27, 1.33, 1.35, 1.35, 1.39, 1.41, 1.43, 1.47, 1.49, 1.49, 1.59, 1.59, 1.67. Mean, 1.27.

The difference between the 2 groups of results seems significant.

No correlation was found between inulin clearance and plasma concentration of inulin or rate of urine flow.

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[2] Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 695.

[3] Holten, C., and Rehberg, P. B., *Acta Med. Skand.*, 1931, **74**, 479.

The concentrations of inulin in the urine were astonishing: in one experiment 35.8%; in another 32.3; in another 22.4. In one instance the concentration ratio, U/P, was 150; in another 132.

It must be stated that in more than half the experiments undesirably large variations in the plasma concentrations of the substances studied (particularly of inulin) occurred, due presumably to the methods of administration. Exclusion of these from consideration does not, however, alter the conclusion drawn from the series as a whole, *viz.*, that the plasma clearance of injected inulin is of the same order as that of injected creatinine and higher than that of xylose. We are inclined to ascribe this difference to greater diffusion of xylose than of inulin from the renal tubule.

One main purpose of this publication is to call attention to the possible usefulness of inulin in connection with renal studies.

7565 C

Occurrence of Non-Motile Leucocytes.

M. SCHWEIZER. (Introduced by Eric Ponder.)

From Washington Square College, New York University.

Sabin, Cunningham, Doan and Kindwall[1] observed that when supravital counts were made on blood drawn every 15 minutes there appeared "showers of non-motile cells" at approximately hourly intervals. They interpreted these as being due to degenerating polymorphs which were actually dying in the blood stream. Beard and Beard[2] confirmed the existence of these showers, and were able to increase their magnitude without altering their rhythm by the injection of sodium citrate. More recent workers (Smith and McDowell,[3] Jones, Stephens, Todd, and Lawrence[4]) have been unable to confirm these observations, and find that the non-motile cells occur at irregular intervals and are probably artefacts.

If the non-motile cells are really dying polymorphs, most of them should be old cells, as determined by the criterion of the number of lobes in the nucleus (Cooke and Ponder[5]), whereas if they are

[1] Sabin, Cunningham, Doan and Kindwall, *Johns Hopkins Hosp. Bull.*, 1925, **37**, 14.

[2] Beard and Beard, PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 614.

[3] Smith and McDowell, *Arch. Int. Med.*, 1929, **43**, 68.

[4] Jones, Stephens, Todd and Lawrence, *Am. J. Physiol.*, 1933, **105**, 547.

[5] Cooke and Ponder, The Polynuclear Count, 1927, London.

artefacts, we might well expect both young and old cells to be affected, *i. e.*, cells of all the classes of the polynuclear count. I have made a series of counts to determine (a) whether the non-motile cells occur regularly, and (b) whether they are always old cells, *i. e.*, cells of the higher classes of the polynuclear count. For each count 2 preparations were made under separate coverslips but on the same slide, and these were examined by the supravital technique, counts of non-motile cells being made in regions of the preparations which initially showed many motile forms. Table I shows a typical series of results. The preparation designated by (a) was the one counted first.

TABLE I.

Preparation	Total cells counted	Polymorphs, %	Non-motiles, %
1 a	100	72	12
b	100	73	4
2 a	100	73	1
b	150	67	9
3 a	150	74	8
b	200	74	3
4 a	100	73	2
b	150	76	14
5 a	200	75	17
b	200	73	4

The nuclei of the non-motile cells became swollen, and the lobation is often obscured: it is possible, in fact, to count the nuclear lobes in only about half the cases. The data on this point are, in consequence, purely qualitative, but where it was possible to distinguish the number of nuclear lobes it was found that there were generally 2 lobes, sometimes one or 3 lobes, and only once 4 lobes. Taken together, the results show that the percentage of non-motile cells show large fluctuations even under the same conditions (*i. e.*, there are large differences between the percentage of these cells in 2 preparations made at the same time from the same sample of blood), whereas the percentage of total polymorphs remains very constant, and also that the non-motile cells are not necessarily old cells. It is a little surprising that a larger number of polymorphs of class IV were not seen, but these, the oldest cells of all, may well have been represented among the non-motiles whose nuclear lobation was impossible to determine. It is to be concluded that the non-motile cells are produced by some uncontrollable factor involved in making the preparation, and that the presence of these cells can not be interpreted as indicating the presence of dying polymorphs in the blood stream.

From purely numerical considerations, it is difficult to see how

one could expect to detect the presence of dying cells by any of the known methods of counting even if they were to die in "showers" at hourly intervals. Assuming that there are 7000 polymorphs per mm.^{3} of blood, and that the average life of these cells is about 15 days (Cooke and Ponder[5]), there would be 20 cells per mm.^{3} of blood dying each hour, or only 0.002% of the white cells in a given volume of blood.

7566 P

Simultaneous Excretion of Creatinine and Certain Organic Compounds of Iodine.*†

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In these experiments the urinary excretion of creatinine has been compared with that of (1) mono-iodo-methane sulphonate of sodium (Skiodan), (2) 3:5 diiodo-4-pyridon-N-acetic acid diethanolamine (Neoskiodan, Diodrast) and (3) sodium ortho-iodohippurate (Hippuran). These 3 organic compounds of iodine owe their practical usefulness in excretion urography to their exceptionally rapid elimination in the urine. Of the normal constituents of urine creatinine is excreted in highest concentration relative to the plasma level. The mechanism of creatinine excretion is not entirely clear (Rehberg,[1] Jolliffe, Shannon and Smith[2]); it is therefore interesting to record that the plasma clearances of these organic compounds of iodine may equal or, under certain conditions, considerably exceed the simultaneously determined creatinine clearances. Their excretion is similar on one hand to that of creatinine, on the other to that of phenol red (Marshall[3]).

In human subjects and unanesthetized dogs various grades of

* The expenses of this investigation were defrayed in large part by a grant from the Commonwealth Fund.

† The organic compounds of iodine were supplied by the Winthrop Chemical Company and by the Mallinckrodt Chemical Works.

‡ Determinations of creatinine were made by Miss E. H. Shiels.

1 Rehberg, P. B., *Biochem. J.*, 1926, **20**, 447, 461.

2 Jolliffe, M., Shannon, J. A., and Smith, H. W., *Am. J. Physiol.*, 1932, **100**, 301.

3 Marshall, E. K., Jr., *Am. J. Physiol.*, 1931, **99**, 77.

water diuresis were induced. Creatinine was administered intraperitoneally (1.0 to 1.5 gm.) in dogs and orally (3.0 to 5.0 gm.) in man. Skiodan and Neoskiodan were injected intravenously. Hippuran was administered intravenously in dogs, orally in man. Large amounts of organic iodine were given to dogs but in man the amounts were limited to the ordinary dose used for excretion urography. Urine and samples of blood were collected at half-hourly or hourly intervals.

Urine and separated plasma were analyzed for iodine by Leipert's[4] method, and for creatinine by the method of Folin as used by Holten and Rehberg.[5] The clearances (C) of both iodine and creatinine were calculated in terms of cc. of plasma cleared per minute by the usual equation; urinary concentration (U) divided by blood concentration (B) times the volume (in cc.) of urine formed per minute (V), $C = \frac{U \times V}{B}$.

The excretion of creatinine and organic iodine was independent of the rate of urine formation. Fig. 1 shows the relation between

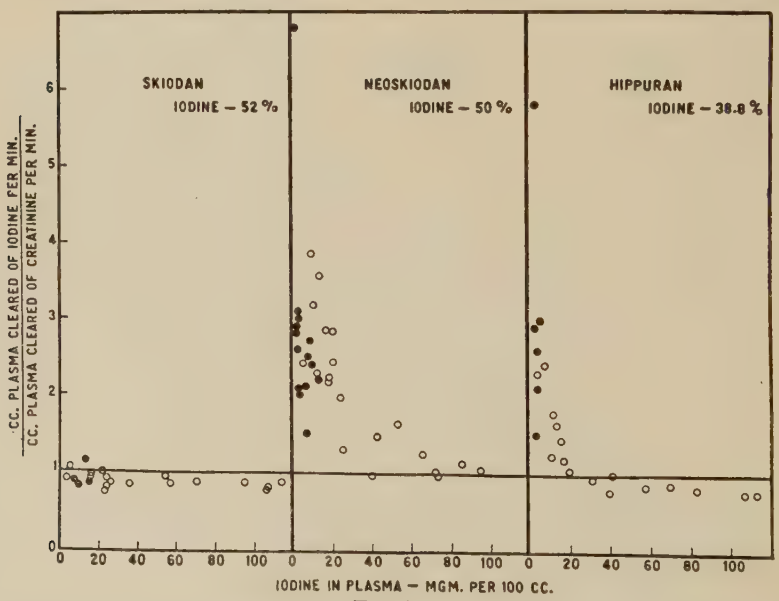

FIG. 1.

Chart showing ratios between plasma clearances of creatinine and of skiodan, neoskiodan and hippuran, in relation to the concentration of each organic compound of iodine in plasma (expressed in terms of iodine). Dots refer to man, circles to the dog.

[4] Leipert, T., *Biochem. Z.*, 1933, **261**, 436.

[5] Holten, C., and Rehberg, P. B., *Acta Med. Skand.*, 1931, **74**, 479.

simultaneous creatinine and organic iodine clearances; the latter for convenience are expressed in terms of iodine alone. Points on the horizontal line, intercepting the ordinate at 1.0, indicate that the clearances of organic iodine and creatinine were equal. The distance by which a given point is above or below that line shows the amount by which the iodine clearance exceeded or fell short of the simultaneous creatinine clearance, *i. e.*, the magnitude of the ratio, $\frac{\text{cc. plasma cleared of iodine per minute}}{\text{cc. plasma cleared of creatinine per minute}}$. This ratio is charted against the average concentration of iodine in plasma during the clearance period.

Skiodan clearances were approximately equal to creatinine clearances over a wide range of plasma Skiodan concentration. Neoskiodan and Hippuran clearances, however, were of the same order of magnitude as the creatinine clearances only when the concentration of Neoskiodan, or Hippuran, in plasma was high. They became several times greater than creatinine clearances when the concentration of Neoskiodan or Hippuran in plasma approached zero.

It appears, therefore, that the mammalian (dog and man) kidney excretes Skiodan and creatinine at approximately the same rate relative to plasma level. Under certain conditions Neoskiodan and Hippuran clearances have the same order of magnitude as the simultaneous creatinine clearances. The mammalian kidney can, however, concentrate Neoskiodan and Hippuran more highly than creatinine when their respective concentrations in plasma approach zero. This relationship provides a new tool for investigating renal function. The mechanism by which the kidney excretes these substances is being studied further.

7567 C

Effects of Deuterium Oxide on Respiration of Germinating Seeds.

GEORGETTE J. MELOT.* (Introduced by J. Ewing.)

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Lewis,[1] the first to report on the biological effects of heavy water, states that tobacco seeds do not germinate in nearly pure deuterium oxide, and that they do so very slowly in water containing 50%

* Fellow of the C. R. B. Educational Foundation.

[1] Lewis, G. N., *J. Am. Chem. Soc.*, 1933, **55**, 3503.

deuterium oxide. In view of the new field of research which this finding would open up, if confirmed, it seemed desirable to obtain a quantitative measurement of the effect of deuterium on germination. For this reason we have made a study of the rate of aerobic respiration of wheat seeds in the early stages of germination.

It is necessary to determine first, whether deuterium oxide inhibits the germination of seeds. If so, does it act as a toxic agent, or if mixed with ordinary water, does it simply dilute the ordinary water until the latter is present in insufficient quantity to support germination? If the deuterium oxide acts as a toxic agent, is its action evident before or after the beginning of karyokinesis?

The respiration of wheat seeds under different conditions was followed by means of a Warburg respirometer. To bring about germination with very small volumes of water, most of the endosperm portion of the seed, whose rate of respiration is low, was discarded, and the experiments were performed with the end of the seed containing the embryo and a small portion of reserve material, it having been found by experiment that this procedure did not interfere with germination in ordinary water.

For each experiment the embryo ends of 10 seeds were placed in the central portion of each of 4 small respirometer (Warburg) vessels. Five hundredths cc. of heavy water were then added to the seeds in each of the 2 experimental vessels and 0.05 cc. of ordinary distilled water to the seeds in each of the control vessels. The seeds were allowed to germinate for different lengths of time at 26°C. Two-tenths cc. of 7% NaOH were then placed in the outer portion of each vessel and the rate of respiration at 26°C. was determined over periods. The conditions of light and O_2 supply were the same for experimental and control series. The experimental error of the method was such that differences between experimental and control figures of less than 10% can not be considered significant.

Since the quantity of heavy water available was small, it was necessary to determine the minimum volume of water necessary to

TABLE I.

Respiration of 20 Embryos in Different Quantities of Water 21 Hours after Beginning of Germination.

Wt. dry embryos mg.	Period of observation, Min.	Vol. O_2 consumed, $mm.^3$ Seeds in 0.1 cc. H_2O	Seeds in 0.15 cc.
102	15	31.5	38.5
	30	64.8	79.4
80	15	31.5	35.0
	30	59.7	68.0
	45	96.4	103.6
	60	118.3	131.8

support satisfactory germination in a given number of seeds. From the figures given below it is evident that 0.1 cc. of ordinary water will cause germination in 20 embryos, but that the rate of respiration is significantly higher with 0.15 cc. than with 0.1 cc.

As it was difficult to know with accuracy the pH of each sample of heavy water used (since the behavior of the deuterium ion towards indicators is not yet well known), it became necessary to test how far the respiratory rate would depend on changes in the pH due to an impurity as traces of NaOH.

TABLE II.
Respiration of 20 Embryos in Solutions of Different pH.

Wt. dry embryos	Age	Period of Observation	Vol. O_2 consumed. Seeds in 0.1 cc. ordinary distilled water pH 5.5	Vol. O_2 consumed. Seeds in 0.0003 N NaOH pH 9.0
mg.	hr.	min.		
78	21½	15	19.7	20.4
		30	55.8	56.5
	34	15	37.3	41.5
		30	76.4	87.7
80	18½	15	22.8	23.4
		30	66.4	65.8
	43	15	35.1	49.6
		30	78.6	107.2

No differences in the respiratory rates of the seeds in the 2 solutions were observed on the first day, but differences began to be apparent on the second day.

Preliminary work was done with 14.8% *deuterium oxide*. A series of 20 embryos were allowed to germinate for 13 hours in 0.1 cc. of ordinary water, another series of 20 embryos in heavy water, and the respiratory rate measured. At this time imbibition was active, but karyokinesis had not begun. An additional 0.1 cc.

TABLE III.
Respiration of 20 Embryos in 14.8% Deuterium Oxide.

Wt. dry embryos	Age	Period of Observation	Vol. O_2 consumed, mm.^{3}. Seeds in 0.1 cc. 14.8 % deut. ox.	Seeds in 0.1 cc. ordinary distilled water
mg.	hr.	min.		
120	13	15	34.3	36.5
		30	66.5	67.8
		45	97.6	101.4
		60	128.2	136.6
	14	0.1 cc. 14.8 deut. ox. and ordinary distilled water added at this time.		
	40	15	35.3	35.2
		30	74.6	74.6
		45	113.0	112.0
		60	157.6	156.7

of water was then placed on the seeds, they were allowed to germinate for another 24 hours, and the respiratory rate was measured again. At this time karyokinesis and visible growth had begun. The results are given in Table III.

No significant difference was observed in the respiratory rates of the seeds in ordinary and heavy water. It is evident from this experiment that deuterium oxide has no pronounced toxic action on the germination of wheat seeds, before or after karyokinesis sets in.

The effect of 38% deuterium oxide was then studied.

TABLE IV.
Respiration of 20 Embryos in 38% Deuterium Oxide.

Wt. dry embryos	Age	Period of Observation	Vol. O_2 consumed mm.^{3} Seeds in 0.1 cc. 38% deut. oxide	Vol. O_2 consumed mm.^{3} Seeds in 0.1 cc. ordinary distilled water
mg.	hr.	min.		
82	16.30	15	29.0	28.6
		30	55.8	59.3
		45	93.5	94.1
		60	120.9	121.8

Table V shows the results of experiments with 94% deuterium oxide. This material had the same pH as ordinary distilled water when measured under the same conditions.

The question arises whether sufficient ordinary water might have distilled from the NaOH solution into the 94% deuterium oxide to cause germination in the seeds. This is unlikely, since the NaOH remained in the Warburg vessels only during the time when respiration measurements were being made, and since the volume (0.2 cc.) of NaOH solution used was small. In order to show more definitely that the germination of the seeds in 94% deuterium oxide was not due to such absorption, dry embryos were placed in the inner portion of the Warburg vessel and 0.5 cc. NaOH in the outer. The seeds did not take up sufficient water to germinate although observed for several days. Furthermore, it was found that seeds germinated readily in 94% deuterium oxide in a closed vessel containing no NaOH solution.

It is evident that, except for the reading made during the first hour of germination when the percentage experimental error is high, there is no significant difference in the respiratory rates of embryos in ordinary and heavy water during the first 2 days of observation. However, on the second day karyokinesis was active. Since 0.1 cc. of 94% heavy water contains only 0.06 cc. of ordinary water, and since we have shown above that the respiratory rate of

TABLE V.
Respiration of 20 Embryos in 94% Deuterium Oxide.

Wt. of dry embryos	Hr. germination	Time of observation	mm³ O_2 consumed Exp. 1 94% A	Exp. 1 Dist. B	Exp. 2 94% A	Exp. 2 Dist. B	Exp. 3 94% A	Exp. 3 Dist. B
mg.		hr.						
80	0*	1st ½	6.9	11.0	2.1	4.1	5.2	8.2
		2nd ½	10.0	8.1	4.3	8.2	7.1	7.8
		2nd	18.5	17.9	17.4	20.5	17.6	20.9
		3rd	22.3	25.6	21.5	22.1	23.0	24.7
		4th	26.0	26.8	24.1	26.7	24.2	26.9
		5th			27.5	26.5	27.0	27.2
		6th					30.6	31.0
	24†	1st ¼	29.4	29.8	.....	.....	31.9	35.9
		2nd ¼	29.2	29.8	57.5	59.2	29.8	27.5
		3rd ¼	34.6	31.5	.....	.....	.....	.....
		4th ¼	30.4	28.3	59.0	57.5	56.4	54.8
	.25*	1st ¼	30.3	36.2	.....	.....	.....	.....
		2nd ¼	30.7	38.5	67.7	80.3	70.8	75.2
		2nd ½			74.7	88.9	79.6	92.0
		3rd ½			78.3	91.8	111.8	139.2
		4th ½			83.6	97.1	164.6	185.2
		5th ½			91.5	106.7		
	48	1st ¼	39.4	40.6			23.5	24.1
		2nd ¼	38.4	38.2			22.9	24.8
		3rd ¼	39.1	42.3			25.5	28.4
		4th ¼	42.5	44.3			23.8	27.3

*0.1 cc. of 94% heavy water and of ordinary distilled water added to the organisms in the experimental (A) and control (B) vessels, respectively. Measurements taken as soon as the apparatus was again in temperature equilibrium.

†Measurements made before any new addition of water.

20 embryos in ordinary water is sensitive to changes in the volume of water available in the range 0.1-0.15 cc., it appears that the heavy water does not act simply as a diluent to ordinary water, but that it enters into the life processes in a manner similar to ordinary water.

Summary. A study has been made of the germination of wheat seeds *(Triticum vulgare)* in 14.8%, 38% and 94% deuterium oxide, and ordinary water for control, under as nearly identical conditions as possible. In all cases the seeds germinated and no macroscopical differences could be detected. No significant differences were observed in the respiratory rates of the seeds in ordinary and heavy water during the first and second days of germination.

The work described in this paper was made possible by the kind cooperation of Dr. H. C. Urey, who provided the different samples of heavy water.

The author wishes to express to Dr. G. Failla and to the members of the Biophysics and Chemistry Departments of Memorial Hospital her appreciation of their assistance during the course of this work.

7568 C

Immunization of Mice Naturally Susceptible to a Transplantable Leukemia.

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The transplantable leukemia designated as line I[1] was started from a spontaneous case of lymphatic leukemia in April, 1929. The line has passed through 441 transfer generations and, by routine technique, has been inoculated in massive doses into 3625 mice of the highly inbred strain C 58; all but one of these died with the leukemic indications characteristic of this particular line of cells. The single survivor, which at no time showed clinical effects of the inoculation, was inoculated in the 77th transfer generation, in December, 1930. In the 3¾ years since that time, the 2925 mice from strain C 58 that have been inoculated with the massive standard dose of cells of line I have all developed leukemia. In the light of this record the natural susceptibility of strain C 58 to leukemic cells of line I appears to be fully established. The present experiments, started in April, 1934, are based on 100% susceptibility to the standard dose.

It has been shown previously[2] that between certain limits, reduction of the dosage lengthens the interval before death, and in the early transfers of line I that were used for these experiments (transfers 27-34, Jan.-Mar., 1930) the 18 mice given doses of 6000-9000 cells did not die with leukemia. However, these mice were not tested for an immunizing effect of surviving the small doses.

In returning to the study of small doses of the leukemic cells of line I, 346 transfer generations later, the virulence of the cells had become considerably enhanced and it now is found that the minimum dose that will kill is reduced to the order of magnitude of 200 cells. In successive dilutions of the massive standard dose the interval before death is progressively lengthened, as previously reported,[2] but a dilution is reached (1/1024th of standard) that permits a few of the mice to survive and as the dose is further reduced the proportion of survivors increases until every mouse survives (1/524,000th of standard). The percentage variation in cell number of different doses of a highly dilute suspension is probably

[1] Richter, M. N., and MacDowell, E. C., *J. Exp. Med.*, 1930, **52**, 823; Potter, J. S., and Richter, M. N., *Proc. Nat. Acad. Sc.*, 1932, **18**, 298.

[2] Richter, M. N., and MacDowell, E. C., *J. Exp. Med.*, 1933, **57**, 1.

very great, yet the consistency of the biological results from different dilutions bespeaks a fair approximation of the actual to the theoretical cell content.

Survivors of small doses were found to be resistant to progressively larger doses until the standard dose (in the order of 80 million cells) was given without harm. In some cases considerable growth of the cells inoculated in the first and second treatment was indicated by large spleens and general sickness followed by recovery, but in most cases there was no clinical evidence of any growth of the inoculated cells.

The results of inoculation with various dilutions and repeated reinoculation of survivors are given in Table I. Numbers in the

TABLE I.

Dilute Doses of Leukemic Cells (Line I) in Mice of Strain C58.
S = Standard dose; dilutions of standard dose indicated by negative exponents of 4; 2-3 weeks between successive doses.

Standard dose not given			Standard dose given		
Treatment	Death	Survival	Treatment	Death	Survival
—1	4	0	S (3¾ years)	2925	0
—2	4	0	—5, S	0	8
—2.5	4	0	—7, S	2	13
—3	25	0	—7, —3, S	0	1
—4	24	0	—7, —5, S	0	2
—5	24	8	—9, —5, S	1	4
—6	25	7	—6, —5, —3, S	0	1
—7	25	37	—7, —5, —3, S	0	12
—8	2	2	—9, —7, —3, S	0	6
—9	9	83	—9, —7, —5, S	1	9
—9.5	0	48	—10, —7, —5, S	0	4
—10	0	8	—6, —7, —5, —3, S	0	2
—11	0	4	—7, —7, —5, —3, S	0	2
			—7, —9, —7, —5, S	0	1
—9, —5	3	5	—8, —9, —7, —5, S	0	2
—9, —7	3	46	—9, —7, —5, —3, S	0	16
—9, —9	2	24	—9, —9, —7, —5, S	0	2
—10, —7	1	7	—10, —7, —5, —3, S	0	3
			—11, —7, —5, —3, S	0	4
			—9, —9, —7, —5, —3, S	0	22
				Total	114

columns headed *treatment* indicate dilutions of the standard dose (S); these numbers are negative exponents of 4, so that —1 indicates a dilution of ¼th and —11, 1/4,194,304th of the standard dose. The interval between successive inoculations was usually 2-3 weeks. Mice surviving doses less than standard (entered in the left hand side of the table) in most cases reappear in the right hand side. Animals listed as survivors of the standard dose have shown no sign of leukemia for at least 16 days; this is 4 times the interval

between inoculation and death of the controls. At present 58 survivors of the standard dose have been under observation for at least 3 months.

Although many variations in procedure prevent any simple statement of the degree of success obtained, the phenomenon of active immunization of mice naturally susceptible to massive doses of a certain line of leukemic cells appears to be established by the 114 mice that have been immunized to resist the standard dose, in contrast to the 2925 non-immunized mice of the same strain that have been inoculated since 1930 with the same standard dose without a single survivor.

7569 C

Vaccination of Rabbits Against Intradermal Pneumococcus Infection.

JOHN A. KOLMER AND ANNA M. RULE.

From the Research Institute of Cutaneous Medicine of Philadelphia.

A vaccine of Type I pneumococcus was prepared by cultivating a highly virulent strain in broth for 24 hours at 37°C. The culture was thoroughly centrifuged, the supernatant broth discarded and the pneumococci suspended in sterile distilled water to give approximately 1000 million per cc. This suspension was heated at 60°C. for one hour, cultured for sterility and preserved with 0.3% tricresol.

Similar vaccines were prepared of Type II and Type III pneumococci.

Results with Type I Vaccine. Six adult rabbits were given 1 cc. of Type I pneumococcus vaccine per kilo of weight by subcutaneous injection every 5 days for 5 doses. One week after the last dose all, along with 2 controls, were inoculated intradermally with 0.2 cc. of 18-hour broth culture after the method of Goodner.[1] The controls developed the typical local lesions, associated septicemia, fever, leukocytic changes, etc., and succumbed 4 to 5 days after the inoculation.

All of the 6 vaccinated rabbits survived. Local lesions of slight to moderate severity developed in all with positive cultures of the edema fluid over 1 to 2 days following inoculation which were thereafter sterile. Daily blood cultures were negative in 5 animals;

[1] Goodner, K., *J. Exp. Med.*, 1928, **48**, 1, 413; 1931, **54**, 847.

in 1 the blood culture was positive 24 hours after inoculation and thereafter negative. Mild to moderate fever with slight leukocytosis developed in all over a period of about 3 days and then reached normal.

Two rabbits were given 2 cc. of the vaccine per kilo every 5 days for 5 doses by subcutaneous injection. When inoculated intradermally one week after the last dose both survived with negative blood cultures throughout. The local lesions were mild with positive cultures of the edema fluid only once 24 hours after inoculation in both animals.

Four rabbits were given 1 cc. of the vaccine per kilo every 5 days for 5 doses by stomach tube and inoculated intradermally 5 days after the last dose. Three developed typical local lesions with the associated septicemia, fever, leukocytic changes, etc., and died about 4 days after inoculation; one developed a local lesion of moderate severity with 2 positive blood cultures but survived.

Four additional animals received 2 cc. per kilo every 5 days for 5 doses by stomach tube. When inoculated intradermally 5 days after the last dose 2 survived after moderate local lesions with 2 positive blood cultures, while the remaining 2 died with severe local lesions, septicemia, etc., about 5 days after inoculation.

Mouse serum protection tests were not conducted because as stated by Goodner and Stillman[2] the 2 factors do not necessarily parallel each other.

Results with Type II Vaccine. Two rabbits were given 1 cc. and 2 additional animals 2 cc. of vaccine per kilo every 5 days for 5 doses by subcutaneous injection. Two were given 1 cc. and 2 additional animals 2 cc. per kilo every 5 days for 5 doses by stomach tube. Five days after the last dose all, including 2 controls, were given an intradermal injection of 0.5 cc. of an 18-hour broth culture in 2 injections closely spaced.

Both controls and all vaccinated animals survived. Among the 4 animals receiving subcutaneous injections of the vaccine the local lesions however, were much less severe. Among the 4 receiving the vaccine by stomach tube the local lesions were approximately equal to the controls.

All animals including the controls gave 1 or 2 positive blood cultures during 72 hours following inoculation but thereafter sterile cultures. Among the vaccinated animals and particularly those receiving subcutaneous injections of vaccine, the fever and leucocytosis were appreciably less than observed with the controls.

[2] Goodner, K., and Stillman, E. G., *J. Exp. Med.*, 1933, **58**, 195.

Results with Type III Vaccine. Two rabbits were given 1 cc. and 2 additional animals 2 cc. of vaccine per kilo every 5 days for 5 doses by subcutaneous injection. Two were given 1 cc. and 2 additional animals 2 cc. per kilo every 5 days for 5 doses by stomach tube. All, including 2 controls, were given an intradermal inoculation of 0.5 cc. of an 18-hour broth culture 5 days after the last dose.

All of the vaccinated animals and one control survived while the remaining control succumbed with a severe local lesion, septicemia and associated fever and leukocytic changes. The surviving control developed a moderately severe local lesion with 2 positive blood cultures. The 2 animals receiving subcutaneous injections of 1 cc. of vaccine developed moderately severe local lesions with 2 positive blood cultures but the 2 receiving the 2 cc. dose developed milder local lesions with sterile blood cultures. The 2 animals receiving the 1 cc. dose of vaccine per kilo by stomach tube developed moderate local lesions and positive blood cultures (1 had 2 and the second 4) but ultimately recovered. The 2 receiving the 2 cc. dose per kilo also had moderate local lesions with 2 positive blood cultures but also survived.

Goodner[1] has reported that one intravenous injection of 10 cc. of an extremely heavy suspension of washed heat-killed vaccine of Type I pneumococcus vaccine protected rabbits against the local and associated lesions and found protective antibody in the blood 5 days later. In only 1 rabbit out of 8 was this immunity found to last for 2 months. Stillman and Goodner[2] have also given 6 rabbits intravenous injections of a heat-killed vaccine containing Type I, II and III in equal proportions. All survived when inoculated intradermally after the last injection, whereas 3 controls died 1 to 4 days after inoculation. Additional rabbits given intravenous injections of pneumococcus autolysate developed a lesser degree of resistance to intradermal infection. The active immunity was found highly specific for the homologous pneumococci and very low to heterologous types.

We preferred to use subcutaneous injections because adapted to practical use in the immunization of human beings. Oral immunization was also employed because previous investigations by Ross[3] have shown this route is effective in rats and we have also found it somewhat effective in the immunization of rabbits[4] and monkeys.[5]

[3] Ross, V. J., *J. Exp. Med.*, 1930, **51**, 585; *J. Immunol.*, 1926, **12**, 219; 237; *J. Lab. and Clin. Med.*, 1927, **12**, 566; PROC. SOC. EXP. BIOL. AND MED., 1926, **24**, 273.

[4] Kolmer, J. A., and Rule, A. M., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 107; 1933, **31**, 245.

Apparently very large doses of vaccine are required by either route of administration and the resulting immunity is apparently of short duration.

Summary. 1. Rabbits have been successfully vaccinated by 5 subcutaneous injections of a heat-killed aqueous suspension of Type I pneumococcus vaccine against fatal intradermal pneumococcus infection. 2. When the vaccine was given by stomach tube about 38% of rabbits survived. 3. With Type II vaccine all animals including the controls survived following intradermal pneumococcus infection but the local lesions, septicemia and associated febrile and leukocytic changes were less marked among the immunized animals and especially those receiving 5 subcutaneous injections of vaccine. 4. All rabbits immunized with Type III vaccine by subcutaneous and oral administration survived along with 1 out of 2 controls following intradermal infection. The local lesions and associated fever and leukocytosis were milder among the vaccinated animals than in the controls and especially among those immunized with subcutaneous injections of vaccine.

7570 C

Chemotherapy of Intradermal Pneumococcus Infection of Rabbits. Effects of Optochin and Other Quinine Compounds.

JOHN A. KOLMER AND ANNA M. RULE.

From the Research Institute of Cutaneous Medicine of Philadelphia.

Rabbits were inoculated intradermally with 0.2 cc. of 18-hour broth cultures of a highly virulent Type I pneumococcus after the method of Goodner.[1]

Immediately after 2 rabbits were given 0.005 gm. *optochin base* (Merck) and 2 additional animals 0.01 gm. per kilo by stomach tube and the doses repeated every 6 hours over a period of 3 days. There was no appreciable influence upon the local lesions, temperatures, leukocytic changes, positive blood cultures or cultures of edema fluid. All animals succumbed in from 4 to 5 days.

Two rabbits were given *optochin base* in dose of 0.01 gm. per kilo every 6 hours for 4 doses before intradermal inoculation with pneu-

[5] Kolmer, J. A., and Rule, A. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 243.

[1] Goodner, K., *J. Exp. Med.*, 1928, **48**, 1, 413; 1931, **54**, 817.

mococcus and then every 6 hours thereafter for 4 additional doses. The compound was without appreciable effect upon the local lesions, associated septicemia, etc., and both succumbed in 4 days.

Two rabbits were given *optochin base* suspended in oil by intramuscular injection in dose of 0.01 gm. per kilo immediately after intradermal inoculation with pneumococcus and repeated every 24 hours for 3 additional doses. Both succumbed about 4 days later with no appreciable effects upon the local lesions, associated septicemias, etc.

Two rabbits were given *ethyhydrocuprein hydrochloride* dissolved in water by intramuscular injection in dose of 0.01 gm. per kilo immediately after intradermal inoculation with pneumococcus and repeated daily for 2 additional doses. Both animals succumbed between 3 and 4 days after inoculation with no appreciable effects upon the local lesions, septicemias, etc.

Two rabbits were given *quinine* and *urea hydrochloride* by stomach tube in dose of 0.01 gm. per kilo immediately after intradermal inoculation with pneumococcus and the dose repeated every 6 hours for 6 additional doses. Two additional animals were given the same compound in the same dosage by intramuscular injection immediately after intradermal inoculation and repeated every 6 hours for 4 additional doses. All 4 animals succumbed in about 4 days with no appreciable effects upon the local lesions and associated septicemias, fever, leukocytosis, etc.

Two untreated controls inoculated intradermally at the same time developed the typical local lesions with daily positive cultures of edema fluids, daily positive blood cultures, leukocytic and febrile changes and succumbed between 4 and 5 days after inoculation.

Summary. Optochin base, ethyhydrocuprein hydrochloride and quinine and urea hydrochloride administered by stomach tube and by intramuscular injection in repeated doses had no appreciable curative effects upon the local lesions, associated septicemia, fever or leukocytic changes induced in rabbits by the intradermal inoculation (Goodner) of virulent type I pneumococcus.

7571 P

Sulphates of Sodium and Magnesium on Gastro-intestinal Activity.

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Our experiments were designed to ascertain the effects of the sulphates of sodium and magnesium on the gastro-intestinal tract of unanesthetized dogs, each having a gastric and intestinal fistula. Rozen and Perussé[1] using unanesthetized dogs with a gastric fistula reported on gastric activity several hours after the use of magnesium chloride but other similar work has not been noted.

Two female dogs, S and B, were used as subjects. The fistulae were made after the manner of Thiry[2] (intestinal) and Carlson[3] (gastric) in a 2 stage operation. The animals were in good physical condition. Food was withheld for 18 hours prior to an experiment, but water was allowed *ad libitum*. Both subjects were trained to lie quietly during the experiments. Our experiments will be described under 2 series since the technic in each differed.

Series I—This series deals with the effects of the sulphates of sodium and magnesium on gastro-intestinal activity when the salines were placed in the stomach, graphic records (smoked paper) being obtained from both stomach and intestinal segment by means of balloons and soap water manometers. The salines were usually given after a normal record of about 1 hour had been procured.

The controls for this series were of 2 kinds: (a) with the stomach empty and (b) with the stomach containing 0.85% sodium chloride.

Results: Isotonic sodium or magnesium sulphate (25 to 50 cc. amounts) in contact with the gastric mucosa was without noteworthy effect on gastro-intestinal activity. Hypertonic (5 times) solutions of magnesium sulphate, however, depressed both gastric and intestinal movements slightly but were without effect on intestinal activity. Hypertonic sodium sulphate (5 times) appeared to increase intestinal activity about 30%.

Series II—Series II deals with the effects of the above salines on the gastro-intestinal activity of dogs S and B when the salines were

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1 Rozen, J. S., and Perussé, G. L., *Am. J. Physiol.*, 1929, **91**, 298.

2 Thiry, *Sitzgsbr d. Wiener Acad. Math. Nat. Hist.*, 1864, **50**, 77.

3 Carlson, A. J., "The Control of Hunger in Health and Disease," University of Chicago Press, 1916, 42.

placed in the loop of the intestinal fistula. As before, simultaneous records of the gastric and intestinal movements were taken, the gastric movements being recorded by the balloon method and the intestinal activity by a new closed system method in which the intestinal fistula was directly connected to the manometer. The essential parts in this system were: fistula—reservoir—manometer. A small stopper carrying a glass tube was placed in the opening of the fistula (dog B) or a rubber tube over a glass tube (dog S). Between the manometer and the fistula was a reservoir into which the solution from the intestinal fistula could flow when contractions occurred and from which the solution could drain into the segment of intestine when relaxation occurred. The manometer was the same type used in the first series. This method of recording intestinal activity we believe is essential, since there is no escape of fluid as in the balloon method, thus making long observations possible.

The results obtained were from experiments of 4 hours duration in which the sulphate solutions were either interspersed between physiologic saline or following it, each saline being left in the intestinal fistula for a period of about 1 hour. The hypertonic solutions were never more concentrated than twice hypertonic. Distilled water was also used in a few experiments.

Results: *Stomach*—no noteworthy effects.

Intestines—Hypertonic sodium chloride and distilled water: Distilled water in general caused only a slight increase in movements, except in 2 experiments in "B" the amplitude was doubled when used following isotonic sodium chloride. Hypertonic sodium chloride increased the amplitude over the normal but slightly in some instances, whereas in others the amplitude was increased 8 times that of the control, the average being about 3 times. There was also a temporary increase for a few minutes in the fluid content of the intestines.

Isotonic sodium sulphate and isotonic magnesium sulphate: The isotonic sodium sulphate stimulated the activity to twice that of the control sodium chloride regardless of whether it preceded or followed the magnesium sulphate. The magnesium sulphate stimulated the activity to 4 times that of the control. When isotonic sodium chloride followed isotonic magnesium sulphate the usual basic effects of isotonic sodium chloride were absent. Both isotonic magnesium and sodium sulphate increased the fluid content of the

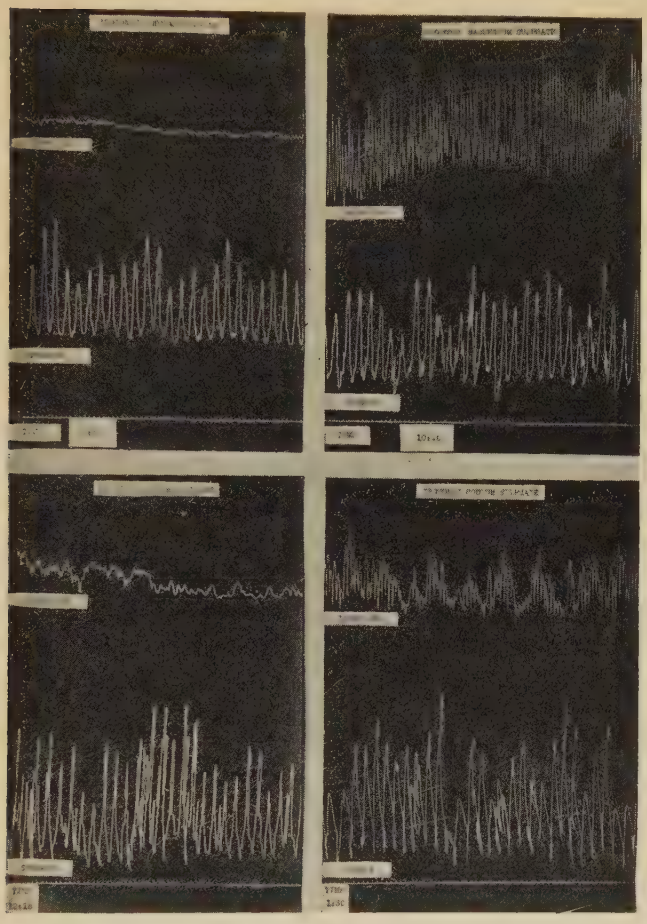

FIG. 1.

Effects of various isotonic salines on gastric intestinal loop activity on unanesthetized dog (Brindle: 12½ kg. Female. 12/22/33.)

Time in 6 seconds: Tracings as follows: Upper left, isotonic sodium chloride; upper right, isotonic magnesium sulphate; lower left, isotonic sodium chloride; lower right, isotonic sodium sulphate.

The upper tracings were taken one-half hour, lower tracings about 45 minutes, after the salines were placed in the intestinal loop.

intestines, the effects lasting throughout the experiment. The magnesium effect was the more marked.

Hypertonic sodium sulphate and hypertonic magnesium sulphate: The magnesium sulphate (twice hypertonic) caused a stimulation of intestinal activity 9 to 10 times that of the control (isotonic sodium chloride). The sodium sulphate (twice hypertonic) increased the activity about 8 times when it preceded the magnesium, and about 12 times when it followed the magnesium.

When isotonic sodium chloride followed hypertonic magnesium sulphate the usual contractions present during sodium chloride were absent in some experiments and present in others. The fluid content of the intestine was markedly increased with each of the hypertonic solutions but slightly more so after the magnesium. The magnesium also appeared to lower the tone of the intestine.

Specific effects of both magnesium and sulphate ions have thus been demonstrated as well as the usual osmotic effects.

7572 P

Determination of Lactic Acid in Presence of Certain Interfering Substances.

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Probably the best technique now available for the determination of lactic acid is that of Friedemann, Cotonio, and Shaffer[1] as modified by Friedemann and Kendall[2] and, more recently, by Friedemann and Graeser.[3] There are certain substances which interfere with the lactic acid determination; these have been listed in the original and some of the subsequent articles. Some of these substances, notably the sugars, may be removed from solution by the copper sulfate-calcium hydroxide procedure.[2] Appreciable amounts of others, however, remain in solution even after this treatment. Important examples of these substances are malic and citric acids, both of which give large yields of bisulfite-binding substances under the conditions used for the oxidation of lactic acid to acetaldehyde. In fact, by some slight modifications of the conditions it is possible to obtain very nearly a quantitative yield of acetaldehyde from malic acid (90 to 93%).[4] The product formed by the oxidation of citric acid is apparently acetone[5] and by the lactic acid method a yield of about 60% of the theoretical may be obtained.[4] However, both malic and citric acids may be quite completely removed from solu-

[1] Friedemann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, **73**, 335.

[2] Friedemann, T. E., and Kendall, A. I., *J. Biol. Chem.*, 1929, **82**, 23.

[3] Friedemann, T. E., and Graeser, J. B., *J. Biol. Chem.*, 1933, **100**, 291.

[4] McChesney, E. W., unpublished observations.

[5] Kuyper, A. C., *J. Am. Chem. Soc.*, 1933, **55**, 1722.

tion by means of basic lead acetate as follows: An amount of solution thought to contain 20-50 mg. of lactic acid and any reasonable amount of the interfering substance is placed in a 100 cc. volumetric flask, and diluted to a volume of about 50 cc. A drop of aqueous phenol red is added, then 10% NaOH is added drop by drop until the solution is neutral followed by one drop excess. A saturated solution of basic lead acetate (Haden) is added in 5 cc. portions until there is an excess; usually 5 cc. is sufficient and a large excess is to be avoided. Zinc ions should be absent from the solution as some precipitation of lactic acid seems to result if they are present. The solution is now diluted to the mark, mixed, and filtered. Lactic acid is determined in the usual way on aliquot portions of the filtrate. With the addition of the $MnSO_4$-H_3PO_4 reagent, a precipitate forms and this causes some bumping during the subsequent boiling but does not seem to interfere otherwise. Table 1 shows the completeness of the separation of lactic and malic acids by this method.

TABLE I.

Mg. lactic acid in 100 cc. vol.	Mg. malic acid added	Mg. lactic acid found
40.8	0	40.8
0	100	0.8
20.4	100	20.3
40.8	100	40.1
81.6	100	78.6
122.4	100	119.4
40.8	20	40.2
40.8	50	40.0
40.8	75	40.2

The average recovery of lactic acid from the mixtures was 98%.

Other substances. Of the other substances reported in the original article [1] to interfere with the lactic acid determination, the following have been found to give precipitates with basic lead acetate when 10 mg. of the substances are present in the final volume of 100 cc.: tartaric acid, citric acid, tyrosine, cystine, and maleic acid. The following have been found to yield precipitates with basic lead acetate when 5 mg. of the substance are present in the final volume of 100 cc.: tartaric acid, citric acid, tyrosine, and cystine (trace). The sugars glucose, fructose, xylose, galactose, and arabinose are of course not precipitated. This method is not likely to be of value in eliminating the various interfering alpha-hydroxy acids since in addition to lactic acid, alpha-hydroxy isovalerianic acid is not precipitated, nor is the acid derived from the deamination of leucine (alpha-hydroxy isocaproic acid).

7573 P

Virulence of Trichinella Spiralis in a Natural and in an Experimental Host.

G. W. BACHMAN AND J. OLIVER. (Introduced by S. A. Koser.)

From the Department of Parasitology of the School of Tropical Medicine of the University of Puerto Rico, under the auspices of Columbia University.

Measurements were made by passing an active strain of *Trichinella spiralis* consecutively through 2 series of white rats and rabbits at regular intervals. In one series (Series A) comprising 14 rats and 10 rabbits, the strain was passed successively from rat to rat and from rabbit to rabbit for a period of 7 months at intervals of 20 days each. The initial dose of trichinous meat was taken from an experimentally infested rabbit, and in each case consisted of a sub-lethal dose, determined previously on another series of animals. At the end of the 20-day period, the rat and rabbit were killed, and a sub-lethal quantity of their trichinous flesh was then fed to a normal rat and rabbit respectively, *i. e.*, the rat flesh to the rat, and the rabbit flesh to the rabbit. This process was repeated until the *Trichinella spiralis* strain had completely died out or could not be detected in the rabbits, which happened after the fifth feeding, but in the white rats, on whom the same technique had been used, the number of worms progressively increased and the worms retained their power to reinfect normal rats after having been passed through rats in succession for nine months.

In the second series (Series B) of 13 rabbits and 22 rats, the same experiments were made as before, except that in this case the initial trichinous meat was taken from a rat instead of from a rabbit. In this series, as in the former experiment, the strain died out in the rabbits at the end of the fifth feeding, while again, in the rats the number of worms per gram of meat continued to increase, and retained their power of penetrating the muscles of new hosts after a period of 11 months.

From this experiment, we may conclude that when the worms invade the natural host they adapt themselves to conditions in the living body and retain their virulence and power to penetrate muscles. They seem to lose virulence and numbers on successive passage through the experimental or unnatural host.

7574 C

Clotting of Plasma in the Absence of Lipoid.

HSIEN WU.

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Cephalin is known to accelerate clotting of blood. In Howell's theory[1] of blood coagulation a thromboplastic substance (or thromboplastein) is liberated from the platelets or tissues when the blood is shed. This thromboplastic substance is supposed to combine with anti-prothrombin (heparin?), setting free prothrombin which is converted by calcium into thrombin. The thromboplastic substance is fat-soluble and appears to be cephalin. The theories of Morawitz[2] and of Bordet[3] are essentially similar to that of Howell. The thrombokinase of Morawitz is only a different name for the same substance as the thromboplastein of Howell, while the cytozyme of Bordet is believed to be a lipoprotein. In Mill's theory[4] of blood clotting, thrombin is a protein-calcium-cephalin compound and the fibrin is a protein-calcium-cephalin-fibrinogen compound. If this view is correct, clotting is impossible in the absence of cephalin, while according to the other theories clotting can still occur in the absence of lipoid, once the prothrombin has been converted into thrombin.

Hardy and Gardiner[5] found that plasma which had been freed from lipoids by extraction with alcohol in the cold clotted normally. This seems to suggest that lipoid is not necessary for clotting. However, this observation of Hardy and Gardiner was only incidental to their study of fat-free serum proteins and they did not mention how the clotting was induced. Their clotting mixture must have contained some lipoid, because the clotting agent, be it serum or thrombin, prepared in the usual way, could not be lipoid-free. It would be desirable to study the clotting phenomenon in the entire absence of lipoid.

Preparation of material. Lipoid-free plasma and serum were prepared essentially according to Hewitt's method[6] described in detail elsewhere.[7] Oxalated plasma was used. In one experiment the oxa-

1 Howell, W. H., *Bull. Johns Hopkins Hosp.*, 1928, **42**, 199.

2 Morawitz, P., *Ergeb. Physiol.*, 1905, **4**, 307.

3 Bordet, J., *Ann. L'Inst. Pasteur*, 1920, **34**, 561.

4 Mills, C. A., and Guest, G. M., *Am. J. Physiol.*, 1921, **57**, 395.

5 Hardy, W. B., and Gardiner, S., *J. Physiol.*, 1910, **40**, lxviii.

6 Hewitt, L. F., *Biochem. J.*, 1927, **21**, 216.

late was removed by dialysis against 0.8% NaCl before the removal of lipoid. The result was essentially the same.

For coagulation experiments, the plasma or serum powder was dissolved in 0.8% NaCl solution to give a concentration of 7%. The solutions were centrifuged to remove any insoluble (denatured) protein.

The tests were carried out as follows: 28 cc. of 0.8% NaCl were mixed with

a. 1 cc. natural serum
b. 1 cc. fat-free serum.
c. 1 cc. 2.5% $CaCl_2$
d. 5 drops of 5% etheral solution of plasma lipoid. This was recovered from the ether-alcohol filtrate in the preparation of fat-free plasma and serum. Most of the lipoid was precipitated when the etheral solution was added to the saline. The precipitate was removed by filtration.
e. 1 cc. 2.5% $CaCl_2$ and 5 drops of 5% etheral solution of plasma lipoid.

To each of the above mixtures 1 cc. of fat-free plasma was added. In a similar series of experiment, natural plasma was used. The results are shown in the accompanying table.

TABLE I.
Coagulation of natural plasma and lipoid-free plasma.

Clotting agent	Natural plasma	Fat-free plasma
Natural serum	+	+
Lipoid-free serum	+	+
Calcium	+	—
Lipoid	—	—
Calcium-lipoid	+	+

+ Indicates clotting.

The clotting of fat-free plasma with natural serum or natural plasma with fat-free serum is to be expected, since in these mixtures all the plasma constituents are present. The clotting of the fat-free plasma with fat-free serum demonstrates that lipoid is not essential for the formation of the clot. However, the fat-free plasma does not clot on calcification as the natural plasma does. Only when both calcium and lipoid are added was the clotting of the fat-free plasma induced. Addition of lipoid alone to the plasma could not induce clotting.

Conclusion. From these observations we must conclude that lipoid as well as calcium is essential for the activation of the thrombin, but once this is formed the presence of lipoid is not necessary.

[7] Wu, H., *Chinese J. Physiol.*, 1933, **7**, 125.

7575 C

Differences in Susceptibility to Ultraviolet Radiation of Paramecium Caudatum and P. Bursaria.

W. W. ALPATOV AND O. K. NASTJUKOVA.

From the Laboratory of Ecology, Institute of Zoology, University of Moscow.

I. In our previous paper[1] we were able to show that the influence of ultraviolet radiation on *P. caudatum* can be expressed by the Arndt-Schultze's law. Since the body of *P. bursaria* is full of green symbiotic algae, it seems worth while to compare the susceptibility of 2 species of Infusoria, differing in body coloration and hence in absorption of radiation.

TABLE I.

Averages and Probable Errors of the Numbers of Offspring of Infusoria in the First Series of Experiments.

Duration of radiation sec.	*Paramecium caudatum*	*Paramecium bursaria*
Control	100.2 ± 1.77	97.6 ± 2.00
40	85.6 ± 1.67	110.4 ± 3.39
80	69.0 ± 1.70	98.4 ± 1.18
160	45.8 ± 1.47	74.0 ± 2.61

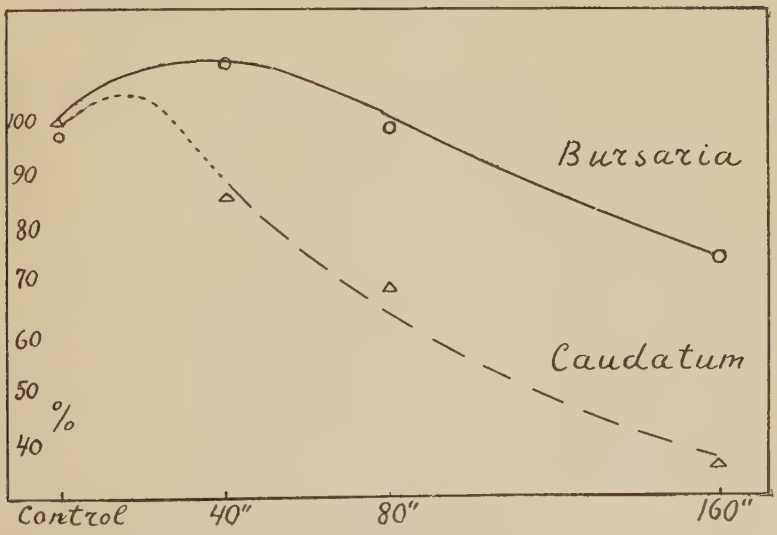


FIG. 1.

Curves showing the relationship between the division rate and the doses of the ultraviolet radiation. The dotted part of the curve for *P. caudatum* is founded on the data of our preceding paper (1933).

[1] Alpatov, W. W., and Nastjukova, O. K., *Protoplasma*, **18**, No. 2.

2. As a source of light was taken a quartz mercury vapour burner of Hereus Hanau, 110 volts of alternating current and 6-7 amp. at a distance of 35 cm. from the animals under quartz Petri dishes filled with water 0.5 cm. deep. The control animals were put under glass dishes to absorb the ultraviolet part of the spectrum. An oatmeal medium containing *Bacillus subtilis* was used. The first series consisted of 5 separate experiments of about 160 specimens each. After keeping them for 24 hours at a temperature of 25°C. on slides with hollows their progeny were counted. The second series of experiments consisted in submitting the same cultures to repeated radiations on consecutive days, keeping the number of parental animals equal to 192. The number of the progeny of each specimen in the first series was expressed in per cent of the average number of the progeny of the control of the corresponding experiment.

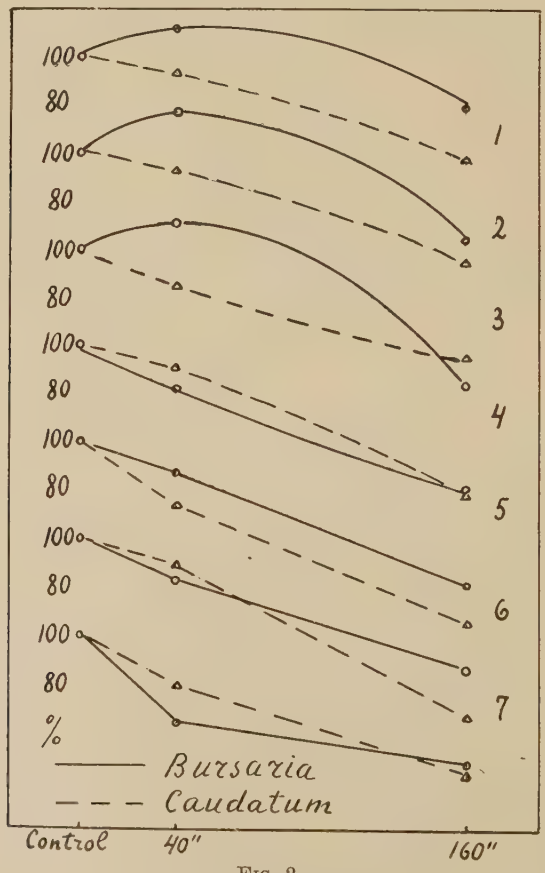


FIG. 2.
Curves showing the results of radiations repeated on 7 consecutive days.

These data show that a radiation of 40 seconds produces in *P. caudatum* a depression of the division rate and the zone of stimulative action is therefore located between 0 and 25 seconds. The curve for *P. bursaria* goes above that of *P. caudatum* and correspondingly the zone of stimulation is extended more to the right somewhere between 0 and 80 seconds. On the whole *P. caudatum* is about 2 times more susceptible to the stimulative and depressive action of the ultraviolet radiation as compared with *P. bursaria.*

The second series of experiments during the first 3 days confirms these conclusions.

Beginning with the fourth day the susceptibility of *P. bursaria* turned out to be practically the same as that of *P. caudatum.* The explanation is as follows: During the whole period of experimentation the cultures were kept in darkness and microscopical observation has shown that *P. bursaria* has lost almost completely the green color of the symbiotic algae. It seems therefore that the presence of pigmented algae is the cause of a greater resistance of *P. bursaria* against the influence of the ultraviolet radiation.

7576 C

Oestrus in Hypophysectomised Rats Parabiotically Connected with Castrates.*

EMIL WITSCHI AND W. T. LEVINE.

From the State University of Iowa.

It is known that unoperated female rats, when united in parabiosis with castrates, first pass through a period of irregular oestral and anoestral activity and later go into a condition of constant oestrus. Hill[1] has reported that during this second period the ovaries always contain large numbers of mature follicles but no corpora lutea. On the contrary in the first period the ovaries are crowded with corpora lutea which, especially during prolonged anoestral phases, are increased in number far beyond anything observed under normal conditions. Cryptorchid males (Martins[2]) as

* This investigation was supported by grants from the Committee for Research in Problems of Sex of the National Research Council.

1 Hill, R. T., *Endocrinol.*, 1933, **17**, 414.

2 Martins, Th., *Compt. Rend. Soc. de Biol.*, 1930, **105**, 789.

well as X-ray sterilized males and females (Levine and Witschi[3]) produce the same effects as castrates (Fig. 6) if put in parabiosis with normal females. This is remarkable since the secondary sex characters are maintained in these cases. An histological examination of the hypophyses proves that the characteristic "castrate cells" are present not only in castrates but also in cryptorchids (Fig. 7) and in X-rayed males and females (Levine and Witschi[3]). Destruction of the germ cells by either treatment causes similar or identical histological and functional changes in the hypophysis.

Fels[4], Kallas[5], and Martins[6] suggested that the known hyper-function of the "castrate hypophysis" might be responsible for the striking reactions in the unoperated parabiont. These authors gave little consideration to the fact that the non-castrate too possesses a hypophysis which may participate in evoking ovarial reactions. The experiments here described will furnish proof that each hypophysis is responsible for one of the 2 main reactions, prolonged anoestrus and constant oestrus.

In a first experiment it was attempted to unite females hypophysectomised for over one month with male castrates. This was not very successful. The females, which were weakened by hypophyseal deprivation, died in all but one case shortly after the operation. In the one surviving case the female recovered to a healthy condition only after several weeks. She remained in anoestrus during the first month of parabiosis and then went into constant oestrus. Obviously, the anoestral phase was due to the poor general condition of the female, following the parabiosis operation. The oestral phase, which has lasted now for 2 months with only one short interruption, must be due to the influx of hypophyseal hormones from the castrate.

A second experiment starts with females that had been in parabiosis with castrate males from 2 to 6 months. All had established the constant oestrus condition. In 5 pairs of this type the hypophysis of the female was removed with no effect upon the oestral type of vaginal smears (Fig. 1, pair 192). It is true that some of the females fell once or twice into anoestrus for periods of 1 to 3 days, immediately following hypophysectomy; though apparently this was due merely to general disturbances caused by the operation.

[3] Levine, W. T., and Witschi, E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1152.

[4] Fels, E., *Arch. f. Gynaek.*, 1929, **138**, 16.

[5] Kallas, H., *Pflüg. Arch. ges. Physiol.*, 1929, **223**, 232.

[6] Martins, Th., *Compt. Rend. Soc. de Biol.*, 1929, **103**, 1341.

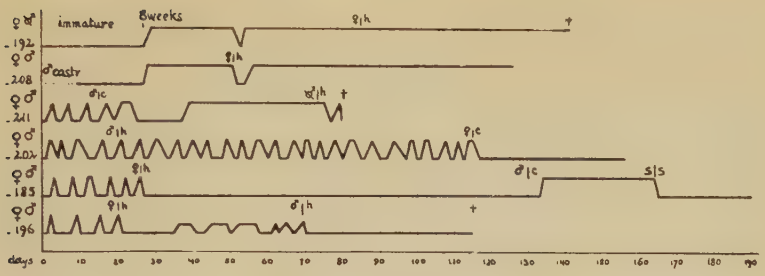


FIG. 1.

Selected oestrus curves of parabiotic pairs of rats. The base corresponds to the anoestral and dioestral condition, the top to full oestrus (stage 2-3). *Pair 192:* male was castrated at birth, united with female at 3 weeks. The female, when 8 weeks old, starts oestrus which is constant with only one interruption of 2 days. Eight weeks later the female is hypophysectomized (♀ h) but remains in constant oestrus. *Pair 208:* Adult male is castrated and united with adult female on same day. No smears taken for first 8 days. Constant oestrus established on 28th day. Female hypophysectomized on 51st day of experiment. *Pair 211:* Normal pair, female has shown regular cycles for some time. Male castrated on 15th day. Female establishes constant oestrus on 39th day. Castrate hypophysectomized on 73d day. Female resumes normal cycles on 75th day. *Pair 202:* Normal pair. Male hypophysectomized on 20th day. Female continues normal cycles until castration on 115th day. *Pair 185:* Normal pair. Female hypophysectomized on 26th day, falls immediately into anoestrus. Castration of male on 130th day brings female into constant oestrus within 4 days. After separation from the castrate on the 164th day the female returns immediately into anoestrus (165th day). *Pair 196:* Normal pair. Female hypophysectomized on 20th day; goes immediately into anoestrus but after the 35th day shows periods of incomplete oestrus. After hypophysectomy of the male complete anoestrus is established again.

Later, constant oestrus is maintained in all cases. (Fig. 1, pair 208).

The ovaries of these hypophysectomised females in constant oestrus are extremely enlarged and contain large numbers of growing follicles (Fig. 2), some of excessive size, others equal to normal mature follicles. New follicles obviously are added constantly while the older ones degenerate (Fig. 2, follicles of irregular shape). These ovaries are larger even than those of non-hypophysectomised females under similar conditions (Fig. 6, Levine and Witschi[3]). The uteri are widely distended and their lumen is filled with cell-debris and leucocytes (Fig. 3). In 4 cases the females were separated from the castrate males 5 or more weeks after hypophysectomy. Within 2 days they fell into anoestrus (compare Fig. 1, pair 185 S,S). Ovaries preserved at different intervals show a rapid atresia of all mature follicles, without ovulation. It is evident, therefore, that the follicular stimulation was due to a hormone received from the castrate.

That this hormone originates from the hypophysis of the castrate is definitely shown in a third set of experiments in which the castrate is hypophysectomised (2 cases), whereupon the unoperated

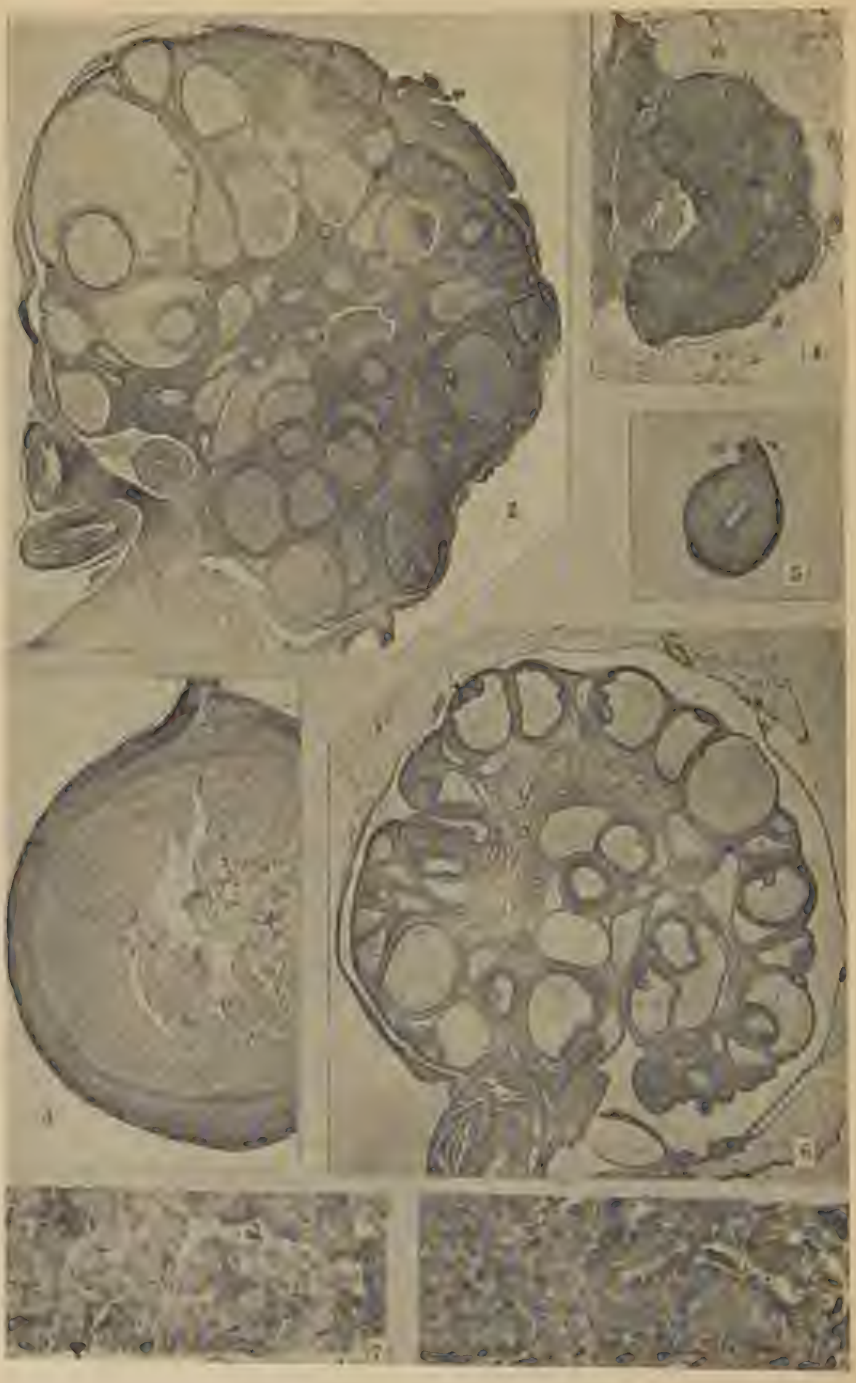


FIG. 2.

Parabiotic pair 186. Ovary of a hypophysectomized female, united with castrate male, after constant oestrus of 5½ months. × 12.

FIG. 3.

Parabiotic pair 210. Similar conditions as in previous case. Cross-section of uterus. × 12.

FIG. 4.

Parabiotic pair 205. Ovary of a hypophysectomized female united with normal female. Preserved after 2½ months of anoestrus. × 12.

FIG. 5.

Same pair as Fig. 4. Uterus of hypophysectomized female. × 12.

FIG. 6.

Parabiotic pair 175. Ovary of female in parabiosis with X-ray sterilized female. Preserved after 3½ months of constant oestrus. × 12.

FIG. 7.

Parabiotic pair 178. Anterior lobe of hypophysis of the male twin, one-half year after experimental cryptorchidism had been established. Note the large "castrate cells" filled with colloid. × 200.

FIG. 8.

Same pair as Fig. 7. Anterior lobe of hypophysis of the female twin, which had been in constant oestrus for more than 4 months. Nearly normal histological picture. × 200.

female returns from the constant to cyclical oestrus (Fig. 1, pair 211). Normal cycles are maintained also by females in parabiosis with hypophysectomised females or males (Fig. 1, pair 202).

The reported experiments show clearly that the condition of constant oestrus is due to an influx of follicular growth stimulating hormone which is released in great quantities by the hypophysis of the castrate co-twin. There remains to be ascertained where the luteinizing hormone comes from, which causes the extensive formation of corpora lutea and the prolonged phases of anoestrus during the first period after castration. The case 211 (Fig. 1) may illustrate once more the sequence of typical reactions that follow upon castration of the male twin. The female still runs one normal cycle, then a prolonged cycle and then falls into a period of anoestrus. Constant oestrus is established only during the fourth week. In more than 20 similar cases Hill (1. c.) and the present authors have found that the period of irregularity preceding constant oestrus can last from 3 to 18 weeks. So, evidently, the hypophyseal system which produces the luteinizing hormone subsists slowly under the influence of the continuous stream of follicle stimulating hormone. Evidence that the hypophysis of the unoperated female is the source of the luteinizing hormone comes from a set of experiments in which we observe that constant oestrus follows castration of the male within 4 or 5 days, if the female had been hypophysectomised 5 to 15 weeks previously. This experiment (4 cases) runs in the following way.

We start with normal pairs (Fig. 1, pair 185). After normal cycles of the female had been observed for some time she is hypo-

physectomised. Immediately she falls into anoestrus, though in some cases she recovers partly, showing irregular and incomplete oestral changes in the vaginal smears. That this activity is due to stimulation by the normal male parabiont can easily be demonstrated. Subjecting him to hypophysectomy brings the female into permanent anoestrus at once (Fig. 1, case 196). Even if the vaginal smears show constantly the anoestral condition, as in case 185, the ovaries are slightly larger than in single hypophysectomised females or in females of double-hypophysectomised pairs. These ovaries contain large numbers of small egg follicles (white dots in Fig. 4) which later undergo fibrous degeneration. No luteinization is observed, and the output of oestrin must be negligible, as indicated by vaginal inactivity and reduced size of uteri (Fig. 5). If now, after an anoestral period of 5 to 15 weeks, the male parabiont is castrated, then the increased amount of hypophyseal hormones coming in from the castrate immediately causes a rapid growth of the Graffian follicles and constant oestrus is established within 4 or 5 days (Fig. 1, case 185).

This quick and clear cut reaction of hypophysectomised as compared with normal females makes it quite certain that in the latter the mainly anoestral period preceding constant oestrus is due to the production of luteinizing hormones by the female hypophysis. As long as this lasts, excessive corpus luteum formation will result because of the simultaneous stimulation of follicular growth by the castrate hypophysis. However the maintenance of a constant and high concentration of follicle stimulating hormone in the blood stream tends to suppress the production and the release of the luteinizer. Nevertheless the hypophysis of the female in constant oestrus retains the potency of producing the luteinizer. For such females, if separated from their castrate twins, resume cyclical changes and may become pregnant and have litters again. Histologically the hypophysis preserves the normal appearance even after months of constant oestrus (Fig. 8). The fact that the two gonadotropic hormones of the hypophysis thus act as an antagonistic pair probably plays an important rôle in the establishment of normal oestrus cycles.

Conclusions. Our experiments with parabiotic rats corroborate the contention of Zondek[7], and Hellbaum[8] gained from entirely different sources of evidence, that the castrate hypophysis emits an increased quantity of follicle stimulating hormone but no luteinizing

[7] Zondek, B., *Arch. f. Gynaek.*, 1930, **144**, 133.

[8] Hellbaum, A., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 641.

hormone. Furthermore we conclude that the maintenance in the blood stream of a constant and relatively high level of follicle stimulating hormone suppresses the production of luteinizing hormone by the hypophysis.

7577 P

Nervous Control of Thyroid Activity. I. Effect of Pilocarpin and Adrenalin on Metamorphic Action of Thyreoactivator.

EDUARD UHLENHUTH, EDGAR VAN SLYKE AND KARL MECH.

*From the University of Maryland Medical School.**

In the experiments reported here we have used amphibian metamorphosis (in the larvae of *Ambystoma tigrinum*) as a physiological sign of the effects of the thyroid hormone, pilocarpin and adrenalin as stimulators of the parasympathetic and sympathetic nervous system respectively, and injections of thyreoactivator from the anterior lobe of the beef hypophysis[1-4] as an activator of the thyroid function.

When pilocarpin or adrenalin alone is injected intraperitoneally, no visible effect on metamorphosis is obtained. It will be shown here that the injection of either of these drugs together with thyreoactivator increases the sensitivity of the larvae to the metamorphic action of the thyreoactivator.

In one representative experiment (CCCLVI, 1933) a number of the larvae of the tiger salamander were divided into 4 groups: Group "a", controls injected with Ringer solution; Group "b, c and d" received triweekly intraperitoneal injections of thyreoactivator extracted from approximately 30 mg. dried anterior lobe, per animal and injection; group "c" received in addition triweekly injections of 2 mg. pilocarpin-hydrochloride (Merck) per animal and injection; group "d" received in addition triweekly injections of 0.05 mg. adrenalin-chloride, 1:1000 (Parke, Davis Company), per animal and injection.

* Aided by the Weaver Fellowship Fund and Julius Friedenwald Fund of the University of Maryland School of Medicine.

1 Uhlenhuth, E., *Anat. Rec.*, 1926, **34**, 119.

2 Uhlenhuth, E., and Schwartzbach, S., *Brit. J. Exp. Biol.*, 1927, **5**, 1.

3 Uhlenhuth, E., and Schwartzbach, S., PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 149.

4 Uhlenhuth, E., and Schwartzbach, S., PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 152.

Group "a" remained larval for the period of the experiment; Group "b" needed an average of 41 injections of thyreoactivator to metamorphose; Group "c" (pilocarpin) needed an average of 12 injections of thyreoactivator and Group "d" (adrenalin) an average of 20 injections of thyreoactivator.

Further work is in progress to show whether the sensitizing effect of pilocarpin and adrenalin is due to a stimulation of specific thyroid secretory nerves or to other effects.

7578 P

Effects of Estrin upon Gonads, Mammary Glands and Hypophysis of the Rat.*

S. R. HALPERN AND F. E. D'AMOUR. (Introduced by I. E. Wallin.)

From the Department of Anatomy, School of Medicine, University of Colorado, and Research Laboratories, University of Denver.

It is generally agreed that injections of estrin cause an atrophy of the gonads[1] and an increase in the weight of the hypophysis with a decrease in its gonad-stimulating power.[2] Wade and Doisy,[3] however, state that Theelin, in dosages up to 6.6 gamma daily, in the male does not cause an interruption of spermatogenesis, and in the female similar doses do not interfere with normal reproductive processes.

Adult normal males and females and female castrates were divided into 2 series, A and B. In Series A, 5 R.U. of estrin[4] were given daily for 3 weeks and 20 R.U. daily the fourth week. In Series B, the same dosage was given as in Series A but injections of 20 R.U. daily were continued for 4 weeks more. Each series included 3 groups with 10 rats in each group: 1, normal males, 2, normal females, and 3, ovariectomized females. An adequate number of controls was used.

Results. I. Effect of estrin on body weight. Normal males and

* This investigation was aided in part by a grant from the National Research Council, Committee on Problems in Relation to Sex.

[1] Moore, C. R., and Price, D., *Am. J. Anat.*, 1932, **50**, 13.

[2] Leonard, S. L., Meyer, R. K., and Hisaw, F. L., *Endocrinology*, 1931, **15**, 17.

[3] Wade, N. J., and Doisy, E. A., *Abst. Proc. Am. Fed. Biologists*, 1934.

[4] Estrin prepared from pregnancy urine according to the method of D'Amour, F. E., and Gustavson, R. G., *J. Pharm. and Exp. Therap.*, 1930, **40**, 4.

castrate females showed a progressive loss of weight while normal females maintained their weight.

II. Effect of estrin on sex organs. A. Male. The testes in Series A weigh only 65%, those of Series B only 23% of the control weights. In the animals of Series B no spermatids or spermatozoa were found, and the tubules contained no secondary, and only a few primary spermatocytes. However, numerous mitotic figures were present in the spermatagonia. The interstitial tissue was considerably reduced. The last breeding in which females were impregnated with these males occurred 19 days after commencement of the injections.

B. Female. The ovaries in Series A are 66% and those of Series B 28% of the control weights. The uterus of the normal female showed a progressive increase in diameter and weight. The uterus of the castrate was restored to approximately the size of the normal female. There was, however, considerable variation in size of the individual uteri and the measurements are not entirely accurate because of contractions and consequent thickening. The weight cannot be used for comparison in this group because at ovariectomy a variable length of the uterine horn had been removed along with the ovary.

III. Effect of estrin upon the hypophysis. In the male and castrate female of Series B the hypophysis is approximately 100% heavier than that of the control weights. In the normal female the hypophysis is about 200% heavier than the control.

Histologically, the pituitaries of females who were ovariectomized 8 months before and injected with estrin for 8 weeks contain very few castration cells. These cells never equalled in size the large "signet ring" cells seen in the untreated animal.

In the anterior pituitaries of normal males and females that received estrin there was an increase in the number of chromophobe cells with a corresponding increase in the number of cells transitional between chromophobes and basophiles. Both the "transitional" cells and the basophiles contain a markedly hypertrophied Golgi apparatus and nucleolus, and numerous enlarged mitochondria, all of which suggest abnormally secreting cells. Many "degranulated" basophiles are to be noted. The connective tissue and vascular elements are also increased.

The pituitaries of Series A resemble somewhat the pituitary of pregnant animals confirming Baniecki's[5] observations on the guinea pig.

[5] Baniecki, H., *Arch. f. Gynak.*, 1928, **134**, 693.

All animals in Series B possessed actively secreting mammary glands. The extent of this development as far as the size of the gland is concerned was greatest in the males, somewhat less in the ovariectomized females, and least in the non-castrate females. A white, fatty fluid (milk?) flowed freely from cut portions of these glands. Histological examination showed a flattened epithelium, prominent vacuolization and alveoli distended with fluid containing many fat droplets and numerous free vacuolated cells.

The effect of long continued injections of estrin upon the mammary glands and hypophysis suggest that the hypophysis is functioning abnormally. Whether this lactation is the result of estrin administration *per se* or whether it resulted after the cessation of the injections is not known, as the animals were sacrificed 4 days after the last injections. This question is now being investigated.

7579 C

Fibrinolytic Activity of Hemolytic Streptococci on Blood of Cases of Recurrent Tropical Lymphangitis.

P. MORALES-OTERO AND A. POMALES-LEBRON. (Introduced by A. M. Pappenheimer.)

From the School of Tropical Medicine, University of Puerto Rico, under the auspices of Columbia University.

Tillett and Garner[1] have recently shown that broth cultures of hemolytic streptococci of human origin rapidly dissolve normal human fibrin clot. Tillett, Edwards and Garner[2] demonstrated the development of resistance to dissolution in the plasma clot obtained from individuals following acute hemolytic streptococcus infections. They also showed that this antifibrinolytic property is absent in the fibrin clot derived from a group of patients convalescing from other infections. Likewise, the blood from the great majority of healthy adults and from persons with other acute diseases was found to be susceptible to fibrinolysis. The authors believe that this insusceptibility to dissolution is specifically induced and that "the fibrinolysin of hemolytic streptococci, in the body, makes a definite response directed against the lytic action of the bacteria."

While studying the probable relationship of hemolytic strepto-

[1] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

[2] Tillett, W. S., Edwards, L. B., and Garner, R. L., *J. Clin. Invest.*, 1934, **12**, 47.

cocci to recurrent tropical lymphangitis, we have made a series of fibrinolytic tests with the blood of a group of patients suffering from this condition. In performing the test the method employed was that recommended by Tillett, Edwards and Garner[2] with the exception that we used tryptic digest broth instead of glucose broth to grow the streptococci.

Thirty-three strains of hemolytic streptococci isolated from different conditions were tested for their fibrinolytic activity on the plasma of a normal individual. All determinations were made at the same time, by the same person, and under similar conditions. The results are shown in Table I.

TABLE I.

Fibrinolytic Activity of Strains of Hemolytic Streptococci from Different Sources.

Strain No.	Source	Complete dissolution in hrs.
*N.Y.5	Scarlet fever	None†
M_4	Sore throat	4 hr.
S_2	Septicemia	8 "
*T	Tonsils	2 "
M_5	Sore throat	5/6 "
S_1	Septicemia	3½ "
T_5	Tonsils	3½ "
O_1	Osteomyelitis	2¼ "
T_1	Tonsils	12 "
T_5	"	2½ "
A_{10}	Meninges	2½ "
A_8	Abscess	5/6 "
A_3	Lesion on knee joint	2½ "
A_4	Pustule	4 "
T_6	Tonsils	5 "
T_3	"	8 "
M_2	Sore throat	20 "
T_8	Tonsils	2/3 "
T_{10}	"	2½ "
A_5	Deep chronic lesion on face	1¼ "
T_4	Tonsils	10 "
T_{11}	"	4 "
A_{11}	Pustule	2 "
*E	Erysipelas	2/3 "
L_{14}	Lymphangitis	1 "
L_6	"	1 "
L_{12}	"	1¼ "
L_5	"	5 "
A_{12}	Impetigo lesion on leg	3 "
L_7	Lymphangitis	3½ "
L_9	"	Partial dissolution in 24 hr.
L_{11}	"	2 hr.
L_8	"	4½ "

*Kindly sent to us by Dr. A. F. Coburn.
†None in 24 hrs.

From these, 2 strains, Nos. T_8 and E, were selected for carrying out the fibrinolytic determinations. The results are shown in Table II.

TABLE II.
Fibrinolytic Determinations.

Case	Condition		Strains	
			E_1	T_8
			Complete dissolution in	
A.P.	Normal		1 hr.	2 hr.
M.E.M.	"		⅔ "	⅔ "
L.G.	"		1¼ "	⅔ "
E.R.	"		1 "	⅔ "
J.M.	"		⅔ "	2 "
M.N.	"		½ "	⅔ "
T.V.	"		2 "	⅔ "
G.V.	Typhoid	Convalescent	3½ "	1⅔ "
C.A.	"	3rd week	1⅔ "	1⅔ "
A.S.	"	" "	Clot retraction	1½ "
A.C.	"	1st "	None†	7 "
N.C.	Thrombophlebitis	Acute attack	2½ hr.	1¼ "
J.G.	Common cold	5 days after attack	2 "	1⅔ "
Z.C.	R. lymphangitis	2 mo. after attack	12 "	5 "
R.A.	Lymphangitis	Attack subsiding	None†	None†
J.L.	R. lymphangitis	24 hr. after onset	None†	None†
M.R.	" "	2 " " "	14 hr.	8 hr.
P.L.	" "	24 " " "	None†	None†
M.R.	" "	1 " " "	6½ hr.	22 hr.
P.L.	Lymphangitis	2 days " "	None†	None†
M.R.	R. lymphangitis	24 hr. " "	None†	None†
F.J.	" "	3 days " "	None†	None†
J.C.*	" "	4 " " "	⅔ hr.	⅔ "
J.P.*	" "	6 " " "	½ "	½ "
F.M.	R. lymphadenitis and lymphangitis	9 " " "	None†	None†

*In these 2 cases, pure cultures of hemolytic streptococci were isolated from local lesions at the time determinations reported were made.
†None in 24 hrs.

Repeated determinations at varying intervals were made in several cases of recurrent tropical lymphangitis. In 4 cases the plasma clot exhibited maximum resistance from the onset of the acute attack to 6 weeks after the attack. In one case, the dissolution time was 14 hours, 2 hours after the onset of symptoms, and maximum resistance on the 18th and 59th day after the attack. Another case exhibited maximum resistance when the attack was subsiding and showed complete dissolution in 10 hours, 8 days after the attack; and in 8 hours, 24 days after the attack.

In 2 cases in which virulent hemolytic streptococci were isolated from local lesions in the affected limb during the acute attack, fibrinolysis was complete in 30 minutes during the attack, and in 2 hours, 8 days after the attack.

In a normal control, where repeated determinations were made at short intervals during 2 months, the dissolution time varied slightly from 30 minutes, the lowest, to 1 hour and 30 minutes, the highest.

Summary: Fibrinolytic determinations made with 33 strains of hemolytic streptococci isolated from different conditions showed individual variations in their lytic activity when tested under similar conditions. Apparently, the plasma clot derived from cases of recurrent tropical lymphangitis develops a definite resistance to the fibrinolytic activity of hemolytic streptococci.

7580 C

Refinements in X-ray Technique for the Estimation of Vitamin D.*

BRIAN O'BRIEN and KENNETH MORGAREIDGE. (Introduced by W. R. Bloor.)

From the University of Rochester, Rochester, New York.

None of the existing techniques commonly employed for the estimation of Vitamin D are entirely beyond criticism. In choosing the most suitable procedure a number of factors must be considered, depending upon whether time and economy or accuracy and reliability are to receive the most consideration. To indicate briefly some of these factors, we have but to point out the principal advantages and limitations of the 3 widely used methods for the assay of antirachitic potency, in all of which inbred stocks of albino rats furnish the experimental animals.

Since all 3 methods may be adapted to either curative or preventive procedures and since the former enjoy by far the greater vogue in this country, the present discussion and experimental work are limited to curative methods. The Steenbock diet No. 2965 was used to produce rickets, and, in general, the recommendations of the Committee of the American Drug Manufacturers Association on Vitamin Assay were followed.[3]

The bone-ash technique as worked out principally by Chick, Roscoe and others[1, 2] is a purely objective procedure, not subject to aberrations of human judgment, even to the extent that they occur in the other 2 methods. On the other hand, the factor of biological variation exerts its greatest influence in this method, and the statis-

* We wish to express our thanks to Dr. Stafford L. Warren and the Department of Radiology of Strong Memorial Hospital for suggestions and the use of X-ray equipment and also to the Department of Biochemistry for animal facilities.

1 Chick and Roscoe, *Biochem. J.*, 1926, **20**, 137.

2 Chick, Korenchevsky, and Roscoe, *Biochem. J.*, 1926, **20**, 622.

3 Holmes, *Rep. A. D. M. A., Com. Vit. Assay*, 1932.

tical significance of the results can be increased only by proportionally lengthening the series of experimental observations in order that the average figures may not be unduly influenced by such factors as variation in animal stature, susceptibility to rickets, etc.

The "line-test"[7] furnishes the most convenient method from the standpoint of time and equipment and can be brought to a high degree of dependability.[3] Here also, however, animal variation may markedly influence the results, particularly variations in susceptibility to the effects of the rachitogenic diet. The only check possible on this point is that obtained by preliminary tests done on random animals before the beginning of the experimental period. Any adequate line-test procedure includes photographing the specimens as soon as the "line" has been developed in order that permanent records may be obtained.[4] A convenient camera for this work was constructed. For the tibia, the negative is made at unit magnification on fine grain film. Prints may be made at magnifications up to 10 diameters without film grain becoming noticeable.

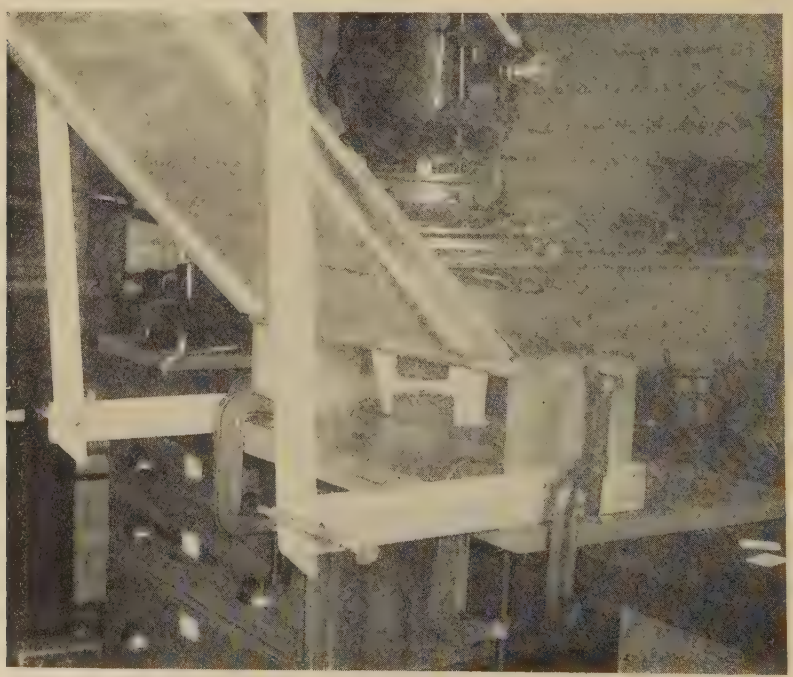


Fig. 1.
Rat and film holder with lead shields.

[7] McCollum, Simmonds, Shipley and Park, *J. Biol. Chem.*, 1922, **51**, 41.
[4] Stevens and Nelson, *Ind. Eng. Chem. Anal. ed.*, 1932, **4**, 200.

One of the first X-ray techniques at all comparable to the line-test was suggested by Poulsson and Lovenskiold.[5] The idea has been further elaborated by Bourdillon, et al.[6] Since X-ray photographs of each individual animal are taken before the beginning of the experimental period, anomalous cases can be recognized and the chief objection to the other 2 methods is overcome, in that variations in susceptibility to the rickets-producing diet do not weight the final results. One serious objection to these techniques, however, lies in the fact that only 2 X-rays are taken, one at the beginning of the experimental period, under anesthesia, and the other at the end of the test after the animals have been killed. While more frequent radiography is desirable, increasing the amount of anesthesia is to be avoided as it may frequently lead to increased incidence of respiratory infection and other metabolic upsets among the experimental animals.

To overcome this difficulty, and to avoid the use of strenuous methods of clamping the rats in position for photographing, which often results in injury, the present modification was developed. Duplitized dental film was held in a special holder (Fig. 1) of such dimensions, and so shielded with sheet lead as to allow both fore and hind legs of the rats to be held in place for exposure with-

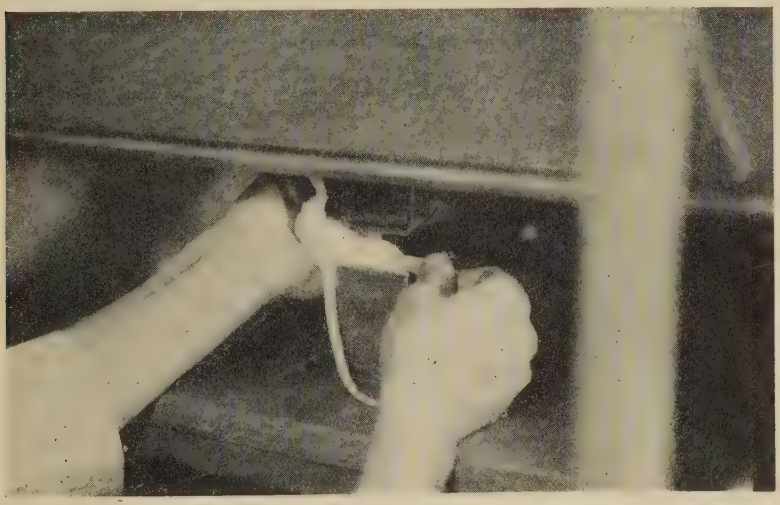
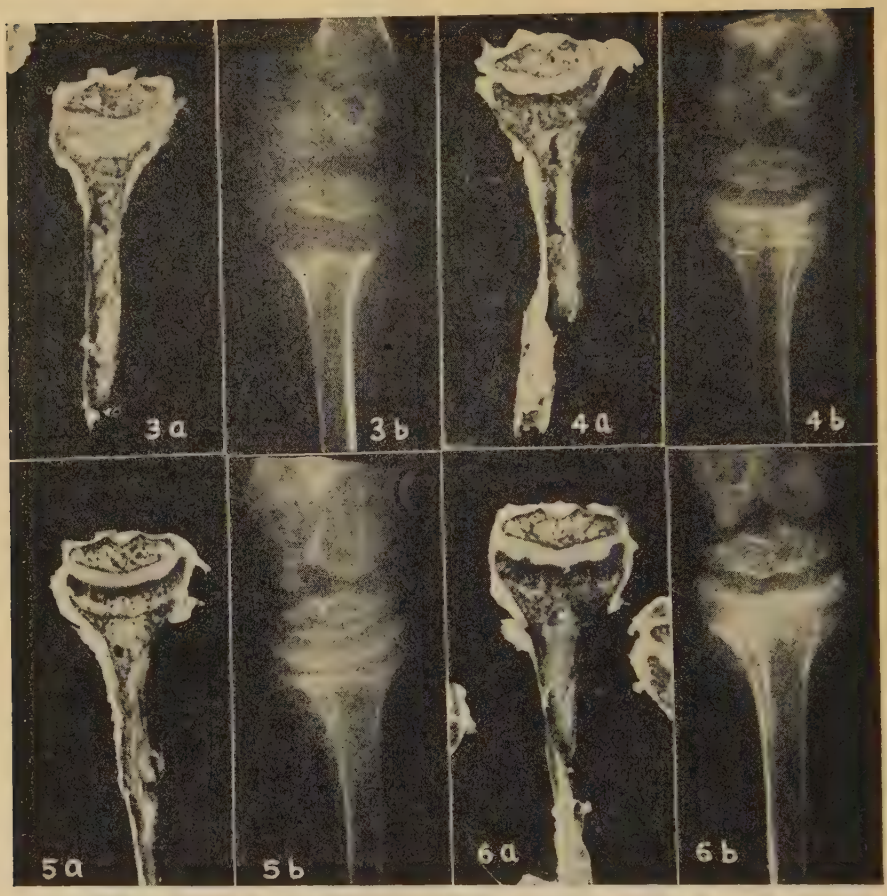


Fig. 2.
Method of holding rat for X-ray photograph of tibial head.

[5] Poulsson and Lovenskiold, *Biochem. J.*, 1928, **22**, 135.

[6] Bourdillon, Bruce, Fischmann and Webster, Med. Res. Council Special Report Series No. 158.



Figs. 3-6.

3 a, b. Line-test and X-ray of rachitic rat.

4 a, b. Line-test and X-ray of healed rickets. Total dose, 16 international units in form of irradiated milk.

5 a, b. Line-test and X-ray showing effect of total dose of 9 units of international standard solution of vitamin D.

6 a, b. Line-test and X-ray showing effect of total dose of 10 international units in form of irradiated milk.

out endangering the hands of the operator. (Fig. 2.) The dental packets were placed in a slide and pushed against a stop. Suitable clips were provided to hold lead stencil numbers for identification. With a little practice, the rats may be held quite easily and loosely without showing any of the fine muscular tremor which accompanies mechanical clamping. There was very little spoilage of film.

We feel that the advantages accruing from the X-ray estimation of Vitamin D by the present technique are obvious. Each animal is checked before the beginning of the test feeding period to estab-

lish the degree of rickets. The progress of healing is followed in each animal at frequent intervals so that any anomalies are recognized. Permanent records are provided and may be re-read at any time. While subjective, the estimations, if based on a comparison of the unknown antirachitic substance with a standard, provide a completely null method of observation. Thus, different observers comparing the X-rays may be expected to arrive at similar results. The averages compare closely with either line-test or bone-ash averages on the same animals, and the spread of observations is certainly no greater than that found with the other methods.

A comparison was made between line-test, X-ray and bone ash estimations on a small series of animals (18 in all) used to determine the potency of an irradiated milk in terms of the International Standard Vitamin D solution. The results (Table I) represent independent estimations by the 3 methods uninfluenced by the other 2. It is felt that the agreement is very good considering the few animals used.

TABLE I.

Feeding Level International Units per day	International Units per cc. of Milk		
	Bone Ash	Line Test	X-Ray
0.5	0.50	0.40	0.40
1.0	0.45	0.40	0.40
2.0	0.50	0.53	0.50
Average	0.48	0.44	0.43

We are indebted to Dr. E. M. Luce-Clausen, who very kindly made the bone ash determinations reported here.

It seems to have been generally recognized among workers in the field, although not, to our knowledge, specifically pointed out in the literature, that the nature of the calcification is by no means independent of the type of antirachitic substance fed. In particular, when an irradiated milk is compared with an irradiated ergosterol concentrate, it has been noticed that the initial calcification is frequently more diffuse in character, "a narrow and continuous line of calcification" seldom occurring in the milk-fed animals.

Examples of the line-test and X-ray photographs on the same animals are shown in Figs. 3 to 6 at equal magnification. Not only the degree but also the character of the healing is shown. In Fig. 5, the narrow line of calcification (animal fed International Standard irradiated ergosterol solution) is shown in marked contrast to the diffuse calcification occurring in an animal fed irradiated milk at substantially the same potency level, Fig. 6.

7581 C

Agglutination of the Encapsulated Anthrax Bacilli.

J. TOMCSIK AND G. BODON.

From the Department of Hygiene, Francis Joseph University, Szeged, Hungary.

There is no convincing experimental evidence on the specific agglutination of anthrax bacilli. The rapid sedimentation of most strains of anthrax bacilli makes a systematic study somewhat difficult. We know however that although by using certain special strains a stable suspension can be secured, the addition of immune serum will not be followed by any particulation, in spite of its containing a high concentration of precipitating antibodies.

A way was opened for the reinvestigation of this question since Szongott and one of us had discovered in the anthrax serum a highly active antibody, hitherto unknown.[1] The antibody of the anthrax immune serum known up to now and used for the thermoprecipitation test acts on the somatic substance of the bacillus, which is a polysaccharide. According to these authors, another and a more potent antibody is obtained, when suitable strains are used for immunization. This antibody reacts probably with the capsular material which is a carbohydrate-free, proteinlike substance. The 2 antibodies were named anti C and anti P respectively.

An accidental observation led us to study the effect of the anti P immune body on the agglutination of anthrax bacilli. We had primarily intended to study the phagocytosis of the encapsulated anthrax bacilli *in vitro*. The strain used for this experiment was isolated of the so called "Carbozoo" vaccine prepared by Mazzucchi and utilized in Italy for prophylactic immunization of animals.[2] This strain was virulent for laboratory animals, it could easily be emulsified in physiologic salt solution and produced in 24 hours an abundant capsule when cultured on agar at 37° C. In this respect this strain appeared very much similar to the "mucoid" varieties, which are obtained when virulent anthrax bacilli are attenuated by repeated subcultures at 41° C. according to Pasteur's well known procedure. When the phagocytosis of this strain was studied in the presence of an immune serum containing both C and P antibodies, a very marked agglutination could be observed under the microscope.

The agglutination was then performed following the usual micro-

[1] Tomcsik, J., and Szongott, H., *Z. f. Immunitätsforsch*, 1933, **77**, 86.

[2] Mazzucchi, M., *La Clinica Veterinaria*, 1931, **9**, 3.

scopic technic. The result was quite surprising when 1:2 - 1:8 dilutions of the immune serum were added. Immediate total agglutination occurred and in an hour all of the bacilli were so firmly attached to each other in a transparent disc, that they could not be separated by intensive shaking. Higher dilutions of the immune serum (1:16-1:256) caused a typical floccular agglutination. This agglutination was strictly specific. No trace of agglutination occurred even when undiluted normal serum was added to the bacterial suspension.

Considering the peculiar nature of the agglutination, we had to weigh the possibility that we might be confronted with a special case of "agglutination by precipitin."[3] This suspicion seemed to us acceptable since we observed that a considerable quantity of P specific substance and a smaller quantity of C substance might be in solution after 24 hours in a broth culture of the encapsulated anthrax bacillus. Consequently we carried out a few agglutination tests with bacilli washed carefully before the performance of the test. No change occurred in the previously recorded results. This reaction therefore had to be regarded as a true agglutination.

The relation of precipitating antibodies to the agglutination. Our previous experiments concerning the antigenic structure of anthrax bacilli had made it very likely that the agglutination of the encapsulated anthrax bacillus had been caused by the same antibody which precipitated the P substance. Direct experiments had to be carried out to prove this supposition.

There was no difficulty in forming an opinion about the rôle of the C antibody since the commercial precipitating anthrax immune serum contains this antibody alone. Four sera were used in this experiment, each of which gave specific precipitation with anthrax polysaccharide up to 1:1,000,000 dilution of the latter and no precipitation whatsoever with P substance. No trace of agglutination was observed by using any of these sera in 1:1-1:1024 dilutions. An entirely negative result was observed likewise by performing this test with a commercial protective immune serum, which contained none of the precipitating antibodies.

On the other hand positive agglutination was obtained with each of the 5 immune sera prepared by us in rabbits through immunization with encapsulated anthrax bacilli. Each of these sera contained an anti C immune body and gave at the same time precipitation with 1:500,000-1,000,000 dilutions of the P substance. Furthermore a certain correlation could be demonstrated between the P precipitin

[3] Jones, F. S., *J. Exp. Med.*, 1927, **46**, 303 ; 1928, **48**, 183.

titer and the agglutinin titer of these sera. The agglutinin titer of the immune sera varied, however, according to the strain used in the experiment.

It was found but once, that the serum of a rabbit bled before the end of full immunization showed agglutination up to a dilution of 1:16, without giving any precipitation with the P substance. The lack of correlation was however temporary since the serum of the same rabbit gave both precipitation and agglutination after 2 more injections. This is another instance that during the immunization the agglutinating property of the immune serum manifests itself earlier than its precipitating property, owing to the fact that different proportions of antibodies are necessary to bring forward the appearance of a visible reaction in the 2 tests.

Agglutination and capsule formation. Two sera were selected for further experiments in order to study the correlation of capsule formation and of the agglutinability in different strains. One of the sera possessed a high anti C and no anti P precipitating activity, the other a high anti P and a lower anti C activity.

Ten different anthrax strains were selected for the first part of these experiments. It was carefully established in wet India ink preparations that none of these strains grown on ordinary agar media ever exhibited a trace of capsule formation. Most of these were virulent, a few attenuated. The bacterial suspensions were prepared by emulsifying a 24 hour agar growth in saline solution and shaking them for varying length of time according to their tendency for sedimentation. In most cases a homogeneous suspension could be obtained though the rate of sedimentation was sometimes increased. The results of the agglutination tests were completely negative, neither of the 2 immune sera caused any agglutination.

An attempt was also made to sensitize these strains through absorption of purified P substance. The bacteria washed and resuspended after this procedure however were just as refractory toward agglutination as before.

On the contrary positive agglutination test was obtained with each of those strains which produced capsules on agar medium. We should emphasize here that not alone strains isolated of anthrax vaccines, or attenuated in our laboratory according to Pasteur's procedure belonged to this group. Almost 50% of our virulent old laboratory strains and some of the recently isolated fresh strains showed some capsule formation. It is true, that in many instances the capsule formation could be detected only by careful search in India ink preparations and extended but to one portion of some of

the long chains. The agglutination by anti P immune serum was without exception positive whenever capsule formation could be revealed by microscopic study. The difference between the agglutination of the virulent strains and the attenuated "mucoid" varieties was that the former gave a floccular type of agglutination, whereas the latter, when lower dilutions of the serum had been used, was agglutinated in the characteristic disc form described above.

We confine ourselves to the description of one strain in order to illustrate the behaviour of the virulent and encapsulated anthrax bacillus. Anthrax strain No. 4 was isolated 6 years ago from naturally infected sheep and kept on agar medium with monthly subcultures. During this time it was transferred only a few times in mice. It was virulent for rabbits. At the time of our study the capsule production of this strain on agar medium was distinct. Its 24 hour agar growth consisted of long chains, some of these completely surrounded by a sharply defined capsule, twice as thick as the bacillary body. Other chains in turn had but a few capsulated members with well defined or with partly dissolved capsules. Some were entirely bare. At this stage this strain gave total agglutination with anti P immune serum up to a dilution of 1:512. The agglutination had a floccular character and contrary to the behaviour of the mucoid strains some inhibition was observed in the lower serum dilutions (up to 1:8). It was then subcultured daily for 15 days. At the end of this period it ceased to produce any capsule on agar medium and its agglutinability was completely lost, that is neither the P nor the C antibody produced any agglutination when mixed to this subculture.

Summary. 1. Antianthrax immune serum containing both P and C precipitin agglutinates only the encapsulated anthrax bacilli. 2. Antianthrax immune serum containing only C precipitin does not give agglutination with any type of anthrax bacilli. 3. P antibody can be regarded as the anticapsular antibody. 4. The serological specificity of the capsules of attenuated "mucoid" strains is the same as that of the virulent anthrax bacillus, if the latter produces capsules on culture media.

7582 C

Effect of Specific Antibody on the Capsule of Anthrax Bacilli.

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The characteristic effect exerted by the type specific immune serum on the capsule of pneumococci, a phenomenon observed by Neufeld, induced several investigators (Sabin,[1] Armstrong[2] and Logan and Smeall[3]) to work out a new rapid method for typing pneumococci. Apart from its practical importance this reaction is of theoretical interest, since it contributes direct proof to the previous supposition that the antipneumococcus immune sera induce in a specific way the alteration of the bacterial capsule. Tulczynska[4] demonstrated that this phenomenon occurs also in case of other encapsulated bacteria, pneumobacillus and streptococcus. Similar experiments performed by her with anthrax bacilli gave however very inconclusive results.

It seems somewhat striking that whereas studies on anthrax contributed perhaps most to the elucidation of the basic facts of immunity, and that the relation of capsule formation to bacterial virulence was first demonstrated with this microorganism, yet our present knowledge on the effect of immune serum on the capsule of the anthrax bacilli is considerably less than in many other bacteria.

Our previous work[5, 6] revealed the existence of an antibody produced by immunization with encapsulated anthrax bacilli and probably acting on the bacterial capsule.

The strain used for the major part of our study produced abundant capsule on agar media. Its 24 hours' growth was very moist and sticky and it killed rabbits with typical symptoms of anthrax infection when a 1/1000 part of a 24 hour agar slant culture was injected subcutaneously. The strain was isolated from the "Carbozoo" vaccine.[7]

Fig. 1 represents a wet India ink preparation showing that almost all of the bacilli are surrounded by well developed capsules.

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[1] Sabin, *J. Inf. Dis.*, 1930, **16**, 469.

[2] Armstrong, R. R., *Brit. Med. J.*, 1931, **1**, 214; 1932, **1**, 187.

[3] Logan and Smeall, *Brit. Med. J.*, 1932, **1**, 188.

[4] Tulczynska, R. E., *Z. f. Hyg. u. Inf.*, 1933, **114**, 769.

[5] Tomcsik, J., and Szongott, H., *Z. f. Immunitätsforsch*, 1933, **77**, 86.

[6] Tomcsik, J., and Bodon, G., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 118.

[7] Mazzucchi, M., *La Clinica Veterinaria*, 1931, **9**, 3.

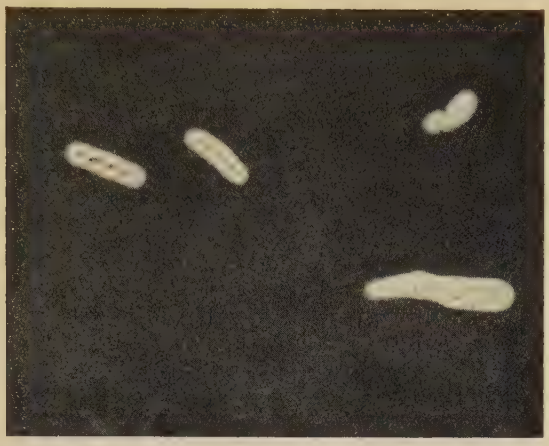

FIG. 1.

No trace of capsule was observed however, when the bacteria were suspended in physiologic salt solution and examined in hanging drop. The result did not differ, when one loopful of different dyes was mixed with the suspension. These observations conform with our previous knowledge, that the capsule of the anthrax bacillus is not visible in hanging drop because its refraction is similar to that of the surrounding fluid.

One loopful of different sera was then added to the bacterial suspension. Several normal as well as non-specific immune sera have been tried, but none of them caused any change in the microscopic picture. The anthrax sera studied here were divided again in 2 groups: (1) sera containing only antipolysaccharide precipitins and

FIG. 2.

(2) those containing both antipolysaccharide and antiprotein precipitins. The effect of 10 different sera belonging to the first group had been studied with completely negative results, that is, the capsules did not turn visible after the addition of these sera. (Fig. 2).

An entirely different picture was observed when we employed 5 sera belonging to the second group. The addition of one loopful of these sera caused agglutination as reported previously. Apart from this the bacteria not included in the large clumps showed a characteristic change. The capsule showed up 1 or 2 minutes after the addition of the serum as a bright body with brown color and with a sharply defined outline toward the fluid. (Fig. 3).

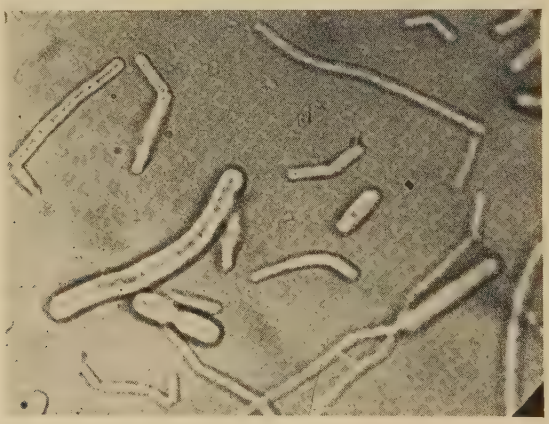

FIG. 3.

The method we adopted finally to show the effect of this immune serum on the capsule was the following: Two loopfuls of the 24 agar culture of the encapsulated bacilli were suspended in 0.5 cc. salt solution. One loopful of this fairly homogeneous suspension was mixed on a cover-slip with the same quantity of Loeffler's methylene blue as well as of the immune serum containing P precipitins. The cover-slip containing this mixture was then inverted and placed over a hollow ground slide and sealed with vaseline. The capsule became visible as a rule after a few minutes owing to its changed refraction. In many instances it was stained as a pink body around the light blue bacilli due to the metachromatic effect of this stain.

In comparing the thickness and the appearance of the capsule with that in the India ink preparation, we had the impression that the visibility of the capsule was not due to a layer of immune serum surrounding and covering it, but rather to a specific reaction which

changed the refraction and the staining properties of the capsule proper.

Altogether 12 different encapsulated and 9 other anthrax strains were examined in this way. We never failed to observe the closest parallelism in demonstrating the capsule production in India ink preparation and in our specific capsular reaction.

The correlation of the agglutinability of our strains and of the capsular reaction was also complete. The capsular reaction therefore has to be regarded as a visible sign of the union of anticapsular antibody with the capsular substance. The agglutination must be in consequence a secondary reaction following the specific alteration of the capsular material.

We believe that it would be of great importance to study in infected animals the protecting rôle of an antibody exhibiting such a marked specific action on the capsule of anthrax bacilli.

Summary. Anthrax immune serum containing P precipitin exerts a specific effect on the capsule of anthrax bacilli. Following the addition of a small quantity of this immune serum to the suspension of encapsulated anthrax bacilli, the capsular material is specifically affected and becomes visible in unstained preparates. This is the final proof of the existence of a separate antibody in specially prepared immune serum acting in a specific way on the capsule of anthrax bacilli.

7583 C

Stability of Toxin Producing Attribute of Scarlet Fever Strains of Streptococci.

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Several reports have appeared concerning the stability of the toxin producing power of scarlet fever strains of streptococci. Organisms dried on swabs or in cultures have been found to retain the toxin producing attribute (Jettmar,[1] Tunnicliff[2]). Tunnicliff[3] reported also that filtrates of certain different appearing colonies of

[1] Jettmar, M. H. v., *Z. f. Hyg. u. Infektionskr.*, 1927, **107**, 265.

[2] Tunnicliff, R., *J. Infect. Dis.*, 1927, **41**, 272.

[3] Tunnicliff, R., *J. Infect. Dis.*, 1931, **48**, 511.

strains which had been cultivated for long periods of time were toxic as were filtrates of recently isolated strains when measured by the paramecium test. However, the filtrates of the dissociated strains were not neutralized by scarlet fever antitoxin. Pilot and Stocker[4] reported a non-hemolytic variant of a scarlet fever strain which was toxigenic. Friedemann and Deicher[5] passed 2 scarlet fever strains 10 times through mice and found that the filtrate of one strain had lost its toxigenic power while in the other strain the toxin producing power had increased. In the work here reported, comparative skin tests were made with filtrates of the original strains and filtrates of these strains after subjecting them to various methods used to dissociate bacteria.

From 3 to 9 strains were used in each method. The original strains had toxin titers ranging from 1:500 to 1:5000. All tests were done on 2 adults who gave consistent results with the various dilutions. Neither of these individuals reacted to injections of toxic filtrates from erysipelas and septic sore throat strains. Dissociation of the strains was attempted by:

1. Subculturing daily in homologous immune rabbit serum diluted 1:10 in infusion broth. These sera were produced by the method used in preparing sera for the dissociation of pneumococcus. The sera contained agglutinins which clumped the homologous streptococcus strains at dilutions of 1:640 or greater. Nine strains were used and the number of transfers varied from 60 to 128.

2. Subculturing daily 4 strains in 1% glucose infusion broth for 60 to 121 transfers.

3. Subculturing daily 3 strains in infusion broth containing methylene blue at a concentration of 0.00001 mol. for 62 to 114 transfers.

4. Subculturing daily 4 strains in infusion broth and incubation at 45°C. for 60 to 119 transfers.

5. Passing 3 strains from 7 to 12 times through mice by intraperitoneal injections and recovery of the organisms from heart blood or peritoneal fluid.

6. From 1 strain 5 atypical colonies were selected which were apparently similar to some of the various dissociated colony forms described by others. Determinations of electrophoretic velocities as well as tests of toxigenicity were made with these strains.

In each instance the toxins, produced by all strains treated by

[4] Pilot, I., and Stocker, S., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 181.

[5] Friedemann, U., and Deicher, H., *Z. f. Hyg. u. Infektionskr.*, 1928, **108**, 192.

these 5 methods, gave reactions to the same dilutions as those of the filtrates of the original cultures.

In the case of the 5 colonies selected from one culture, while all of these produced filtrates which gave reactions at the titer of the original cultures, 3 gave reactions smaller in extent than the other 2. Cataphoresis determinations made on 2 occasions showed that the rate of migration of the organisms producing the smaller reactions was about twice that of the other 2 and of that of original culture. It should be stated, however, that we have previously shown[6] that a faster rate of migration is not a specific characteristic of non-toxin producing strains of streptococcus.

Summary. The use of several recognized methods for dissociation of bacteria when applied to scarlet fever strains of streptococci failed to deprive these strains of their ability to produce toxin.

7584 C

On the Motion of Growth. IX. A Scheme for Analysis of Experiments on Growth, Nutrition and Metabolism.

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The quantitative relationships between normal growth and heat production which the author has recently found and applied in the case of bacterial cultures,[1] in *Bufo vulgaris* from fertilization throughout metamorphosis[2] and from birth to adult life in man[3] should likewise be helpful in dealing with the results of many experimental studies on growth, nutrition, or metabolism.

Such studies are carried out, almost without exception, upon the young of some species, and noteworthily, in the present connection, on subjects still immersed in the "flux of growth." It is just at this stage of life, moreover, that characteristic and often conspicuous changes in metabolism are known to occur. Sufficient evidence in the 3 normal cases we have mentioned has already been brought forward to show that these changes in metabolism, as portrayed in

6 Thompson, R. L., and Megrail, E., *Am. J. Hyg.*, 1934, **19**, 457.

1 Wetzel, N. C., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 360.

2 Wetzel, N. C., *Proc. Nat. Acad. Sc.*, 1934, **20**, 183.

3 Wetzel, N. C., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 227, 233; *J. Pediat.*, 1933, **3**, 252; 1934, **4**, 465.

the data of other workers, are actually due to, and depend uniquely upon, the underlying and concurrent changes in growth itself.

Thus, taking the relation between *growth, q,* and *size, z,* to be, as before,[2, 4] $q = \mu \log_e \frac{z}{z_0}$, with $\mu = 1 = z_0$, we may express the interdependence of growth and metabolism for the case of laboratory animals as follows:

$$\left.\begin{array}{lll} \textit{Growth:} & \lambda\frac{d^2q}{dt^2} + \rho\frac{dq}{dt} + \frac{q}{\kappa} = E & [\text{Cal} / \text{M} / \mu] \\ \textit{Metabolism:} & \rho\left(\frac{dq}{dt}\right)^2 + E_c\frac{dq}{dt} + A' = U & [\text{Cal} / \text{M} / \text{T}] \end{array}\right\} (1)$$

the significance and dimensions of individual symbols having been outlined and applied elsewhere.[2, 4] For present purposes, however, we have briefly: $\dot{q}$, the rate of *growth* as distinguished from the rate of *gain* (less commonly loss), $\dot{z}$; ρ, the resistance, λ, the inductance, κ, the permittance of growth; E, the net external work of growth, and E_c, the work of synthesis, each of the 2 latter constants being referred to the unit of mass (z) and charge of growth (q); and finally, A', the heat of maintenance, in terms of power per unit mass, liberated even when growth is in the state of rest, that is, when $\ddot{q} = \dot{q} = 0$.

These results lead to several suggestions for further experimental work in the fields of growth, nutrition, and metabolism. For the methods which have succeeded in establishing the dynamic connection (1) between the concomitant events of pure growth on the one hand, and those of heat production or metabolism on the other, would now appear to be of considerable assistance in the analysis of various questions arising in these fields when growth itself is directly or indirectly put to experimental test.

How, for example, does a small quantity of lettuce incorporated into the usual diet of young white rats lead to an increase over the "normal" rate of gain?[5] How, also, does the administration of anterior pituitary extract produce a somewhat similar result, the treated rats ultimately weighing about 16% more than the controls,[6] notwithstanding the fact that the quantity of food remained the same in each group, or, even more unexpectedly, in spite of the fact that the fuel value of sacrificed carcasses proved to be less in the test animals than in the controls? The former, it was found, contained less fat. Their tissues possessed, on the whole, the chemical

[4] Wetzel, N. C., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1044.

[5] Outhouse, Julia, and Mendel, L. B., *J. Exp. Zoöl.*, 1933, **64**, 257.

[6] Lee, M. O., and Schaffer, N. K., *J. Nutrition*, 1934, **7**, 337.

constituents recognized as characteristic of younger normal animals; and the changes in body composition usually associated with advancing age, increasing size, and with progress toward maturity, did not take place.

The foregoing combination of results is one of the various possible groups of effects to be expected when growth is artificially distorted; but it also turns out that precisely the combination which Lee and Schaffer describe should likewise be accompanied by decreased heat production. This is of especial interest since these authors have been able to show that the administration of anterior pituitary growth hormone is followed by a drop in metabolism.[6] How, then, have the hormone, food, and growth combined, while acting together, to yield such results?

Other questions are likewise suggested: What initiates the process of growth, or which factors are concerned with the natural acceleration of growth that usually takes place after the onset? What, finally, is responsible in chief part for the fact that growth in healthy structures is strictly confined within "normal" limits, and to what, therefore, is "disorganized" growth in unhealthy tissues to be ascribed?

Questions such as these are not necessarily to be considered as beyond the hope of further analysis or investigation. It should, in fact, be possible to attack them on the experimental side with the promise that definite answers might be obtained in terms of the fundamental parameters set out in equation (1). Let us see how the matter could be expected to work out in practice.

To fix ideas, we assume that an investigator proposes to study several substances or procedures, say $x_1, x_2, \ldots x_n$ by means of their respective effects upon the young of certain species, whose domesticated "normal" rate of growth, $(\dot{q})_0$, may be written, in view of the first of equations (1), in the functional form,

$$(\dot{q})_0 = F[\rho_0, \lambda_0, \kappa_0, (E)_0, \ldots; (q)_0, (\ddot{q})_0] \qquad (2)$$

F, accordingly, being characteristic of the species. Now, $(\dot{q})_0$ will be altered by any one, or suitable combinations of changes in the parameters, $\rho_0, \lambda_0, \ldots$, induced by some external agent, as here, by $x_1, x_2 \ldots x_n$, and we shall have,

$$\left.\begin{aligned} (\dot{q})_1 &= F[\rho_1, \lambda_1, \kappa_1, (E)_1, \ldots; (q)_1, (\ddot{q})_1] \\ &\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \\ (\dot{q})_n &= F[\rho_n, \lambda_n, \kappa_n, (E)_n, \ldots; (q)_n, (\ddot{q})_n] \end{aligned}\right\} (3)$$

F remaining the same since the species is unchanged.

The problems occurring in practice are, however, just the converse of this, for the worker will wish to know which parameters are changed when the normal or control rate of growth, $(\dot{q})_o$, has been experimentally altered. Such a problem is clearly beyond solution, as may be inferred from (1) so long as experimental observations are limited to a study of change in size, z, alone. The best that could be done would be a determination of the ratio ρ/λ, which has the dimension $[T^{-1}]$ simply. But, since $\dot{q}_{(1 \ldots n)}$ can be computed with the help of (1) and (2) from the experimental measurements of size, it is evident that a simultaneous study of heat production will at once permit the numerical evaluation of the individual constants, $\rho_1, \rho_2, \ldots \rho_n$; $\lambda_1, \lambda_2, \ldots \lambda_n$; $(E_c)_1, (E_c)_2, \ldots (E_c)_n$, etc., since the data on heat may be adjusted to the accompanying equations,

$$\left.\begin{aligned} (U)_1 &= \phi[\rho_1, (E_c)_1, (A')_1; (\dot{q})_1, (\dot{q})_1^2] \\ &\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \\ (U)_n &= \phi[\rho_n, (E_c)_n, (A')_n; (\dot{q})_n, (\dot{q})_n^2] \end{aligned}\right\} (4)$$

for substances $x_1, \ldots . x_n$ respectively, the function ϕ remaining the same as long as the observations are taken on the same species of organism. Equations (4), accordingly, provide a method of determining the ρ's and hence, with the latter known, a method for computing all of the remaining constants along with their probable errors if least square procedure be adopted as in the case of *Bufo vulgaris* previously reported.[2] In experiments $x_1, x_2, \ldots . x_n$ we shall obviously meet the various possibilities suggested by the relations:

$$\left.\begin{aligned} &\left.\begin{matrix}\rho_1 \\ \rho_2 \\ \cdots \\ \rho_n\end{matrix}\right\} \gtreqless \rho_o; \quad \left.\begin{matrix}\lambda_1 \\ \lambda_2 \\ \cdots \\ \lambda_n\end{matrix}\right\} \gtreqless \lambda_o; \ \ldots \ \left.\begin{matrix}(E_c)_1 \\ (E_c)_2 \\ \cdots \\ (E_c)_n\end{matrix}\right\} \gtreqless (E_c)_o \end{aligned}\right\} (5)$$

The effect, therefore of any foodstuff, or of any procedure that influences growth may always be estimated in terms of the fundamental properties of growth, ρ, λ, κ, and E, which are held to govern the motion of growth in that particular system. Whether such substances "promote" or "inhibit" growth, their action will become manifest by means of the respective changes which they induce in the normal control values of these 4 parameters of state. The point should ultimately be reached where the more important substances or procedures $x_1, x_2, \ldots x_n$ (*e.g.*, vitamins, hormones or other materials) could be classified with respect to their several or indi-

vidual effects upon the fundamental properties of growth, ρ, λ, κ, E. At that stage it will have become apparent whether a suitable choice of the x's will permit growth to be placed under full control or not.

Summary: (1) Post-embryonic growth of common laboratory animals is governed, in accordance with the first of equations (1), by 4 fundamental properties of growth represented by the constants ρ, λ, κ, and E. (2) Rates of growth ($\dot{q}$) are altered when any one, or suitable combinations of these parameters are changed by experimental means. In practice, however, the problem is more likely to be the converse of this: which parameters are changed when the normal or control rate of growth is known to have been altered? Such a problem is insoluble so long as observations are limited to measurements of change in size, z, alone. (3) Heat production "during growth" ($\ddot{q} \neq 0 \neq \dot{q}$) is quantitatively different from heat production when growth is in the stationary state ($\ddot{q} = \dot{q} = 0$). Heat production per unit time per unit mass is synonymous with metabolism, and the latter is dynamically related to growth *via* the properties represented by ρ and E_c. (4) The values of all constants along with their *P.E.*'s can be computed from simultaneous data on growth and metabolism. (5) The effect of any foodstuff, or of any procedure that influences growth can therefore be estimated in terms of the control values ρ_o, λ_o, κ_o and $(E)_o$, and the substances themselves may be compared by means of the respective changes induced in these four fundamental parameters of state.

7585 C

Effect of Certain Physical Factors on the In Vitro Testing of Anthelmintics.*

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Although certain investigators have done much to destroy confidence in the value of *in vitro* methods of testing anthelmintics by drawing too sweeping conclusions from uncontrolled experiments, these methods are of value and were used successfully by Lamson

*The funds for carrying out this work were given by the International Health Division of the Rockefeller Foundation.

et al.[1] in their studies on the alkyl resorcinols. These studies have resulted in the establishment of hexyl resorcinol as an effective ascaricide of relatively low toxicity. This method of *in vitro* testing of ascaricides is described elsewhere,[2] but there remains for discussion certain physical factors which must be guarded against if reliable results are to be obtained.

As Lo Monaco[3] has already shown with santonin, an undissolved excess of the drug must be present in the testing solution for the best results, but he makes no mention of the effect of the physical state of the excess. However, a solid excess is far less effective than a liquid excess as will be shown. If certain substances, such as heptyl resorcinol, are allowed to stand for sometime in contact with 1,000 parts of 0.9% NaCl solution at 37° C., a part of the chemical will be dissolved, but as these substances are less soluble than 1 to 1,000 a solid excess will remain. If another sample of this same chemical is heated in contact with the same relative amount of 0.9% NaCl and cooled to 37° C. we shall have 2 mixtures of the drug and saline with the sole difference that in one instance the undissolved excess is a solid, while in the other it is a supercooled liquid. When these 2 mixtures are tested against *Ascaris lumbricoides* of swine, it is found that the one with liquid excess will kill the worms in a much shorter time. Table I records the results obtained when several of these substances were tested with both a solid and a liquid excess.

But many solids which have possible uses as anthelmintics will not remain liquid when cooled to 37°. It has been necessary to adulterate these higher melting substances with some organic liquid to keep them in the liquid phase. Many liquids have been tried in this laboratory with that purpose in mind, but usually the dilutant was either toxic to the worms or it inhibited to some degree the activity of the drugs being tested. n-Hexane has served this purpose better than any other chemical as it is non-toxic to the worms and has comparatively little effect upon the drug to be tested. Table II shows the results obtained when solid substances are liquefied with n-hexane.

It is well known that the molecules of a substance in a liquid state are much more motile than molecules of the same substance in a solid state. In the former case they are in no particular relation to one another but may freely move about in the liquid mass,

[1] Lamson, P. D., Brown, H. W., Ward, C. B., and Robbins, B. H., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 191.

[2] Lamson, P. D., Brown, H. W., and Harwood, P. D. In Press.

[3] Lo Monaco, *Arch. ital. de Biol.*, 1896, **26**, 216.

TABLE I.

Drug	Melting point	Exposure necessary to kill *A. lumbricoides* in solid excess	liquid excess
		min.	min.
1-propyl naphthol-2	57°	10	2-5
2-propyl naphthol-1	49°	30-45	10-20
amyl resorcinol	71.5-73°	15	2
heptyl "	73-74.5°	5-10	2
o-phenyl phenol	56°	20-60	5

TABLE II.

Drug	Melting point	Amount of n-hexane	Exposure necessary to kill *A. lumbricoides* with pure drug	adulterated drug
		%	min.	min.
p (3 amyl) phenol	75.5-76°	1/3	20	2
cyclo hexyl resorcinol	128°	1	180	2
p tertiary amyl phenol	93-94°	1	20	2
p tertiary butyl "	97-98°	1	20	2
p chlorothymol	64°	1/3	20	5

while molecules in a solid have certain more or less definite positions relative to their neighbors and are accordingly, more restricted in their movements. A liquid will, therefore, dissolve much more rapidly in another liquid than the same substance in a solid state would dissolve in the same solvent. With these slightly soluble anthelmintics the removal from the test solution of a small amount of the drug would appreciably lower the degree of saturation. With a well agitated mixture this situation would be rapidly remedied if a liquid excess of the drug is present. However, a solid excess with its less motile molecules would be much slower in replacing the removed drug, and the test animals would be exposed to a much lower concentration of the drug. Furthermore, a liquid excess would be able much more readily to penetrate directly into the worm without passing through a dissolved phase than would be the case with a solid excess.

7586 P

Effect of a High Salt Diet on Survival of Adrenalectomized Rats.

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(Introduced by W. W. Swingle.)

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That NaCl is beneficial in the treatment of experimental adrenal insufficiency was established by the earlier work of Stewart and Rogoff,[1] Banting and Gairns,[2] Marine and Baumann,[3] and Corey;[4] and the recent work of Loeb *et al*,[5, 6] Harrop *et al*,[7] Swingle *et al*,[8] Zwemer,[9] and Rubin and Krick.[10]

A consensus of the findings is that NaCl feeding in dogs and cats will prolong, but not indefinitely maintain, life after total adrenal ablation. Rubin and Krick found, however, that in 8 rats a drinking solution of 0.0329% $CaCl_2$, 0.015% MgCl, 0.07% NaCl and 0.035% KCl given upon the appearance of adrenal insufficiency symptoms, would maintain life for 4 months or more in animals which normally would not live longer than 10 days. At the time of their publication these authors had apparently not determined, by discontinuing treatment, whether accessory adrenals had assumed a functional condition. From their work it would appear that the rat, unlike the cat and dog, will live indefinitely if fed a high salt diet after adrenalectomy. At the time of Rubin and Krick's publication we were studying the effects of adding salt to the diets of adrenalectomized rats. In addition to this we adopted their technique of adding salt to the drinking water, a method probably more effective.

In previous experience with our rat colony we found that ap-

1 Rogoff, J. M., and Stewart, G. N., *Am. J. Phys.*, 1928, **84**, 649.

2 Banting, F. G., and Gairns, S., *Am. J. Phys.*, 1926, **77**, 100.

3 Marine, D., and Baumann, E. J., *Am. J. Phys.*, 1927, **81**, 86.

4 Corey, E. L., *Am. J. Phys.*, 1927, **79**, 633.

5 Loeb, R. F., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 808.

6 Loeb, R. F., Atchley, D. W., Benedict, E. M., and Leland, J. J., *J. Exp. Med.*, 1933, **57**, 775.

7 Harrop, G. A., Soffer, L. J., Ellsworth, R., and Trescher, J. H., *J. Exp. Med.*, 1933, **58**, 17.

8 Swingle, W. W., Pfiffner, J. J., Vars, H. M., and Parkins, W. M., *Am. J. Phys.*, 1934, **108**, 159.

9 Zwemer, R. L., *Endocrinology*, 1934, **18**, 161.

10 Rubin, M. I., and Krick, E. T., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 228.

proximately 95% do not survive adrenalectomy.[11] In the latest adult control series 4 out of 24 survived longer than 30 days, 2 longer than 50 days.

In the present experiments, 1.5% NaCl was added to the stock diet* in part of the cases, 2.5% in the others. Either the Rubin-Krick salt solution or 0.9% NaCl solution was given the animals to drink. As far as we could tell one of these feeding-drinking combinations was no more effective than another, so further distinctions between them are not made here.

Twenty-three young, but mature, adrenalectomized rats, weighing from 140 to 200 gm., were studied. The salt treatment was continued for 30 days after adrenalectomy, at which time distilled water and the stock diet were substituted. The results of this treatment from the standpoint of survival can be conveniently divided into 3 categories:

1. Five cases, unlike those of Rubin and Krick, succumbed from 14 to 25 days after operation, *i. e.*, during the course of treatment, with typical symptoms of adrenal insufficiency.

2. Six animals survived and gained weight during the course of the treatment, were apparently in good condition when the treatment was stopped, but after being returned to normal diet developed adrenal insufficiency and died in 10 to 15 days.

3. Twelve animals, approximately 50%, survived in good condition while treated, and after treatment was discontinued gained weight and remained in apparently normal health until killed for autopsy 8 or more weeks after operation. In 7 of these animals accessory adrenals were found.

The survival of 50% after treatment was discontinued is similar to the results we obtained in this colony after withdrawing cortical hormone treatment.[12] Thus it would appear that any agent that will delay the appearance of adrenal insufficiency will, probably by allowing time for the hypertrophy of accessories, cause indefinite survival in this colony in about 50% of the cases.†

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11 Gaunt, R., *Am. J. Phys.*, 1933, **103**, 494.

*Our stock diet is composed of 9 parts by weight of GLF Calf Meal, and one part ground meat and bone scrap. To this is added yeast, cod liver oil, and lettuce.

12 Gaunt, R., and Gaunt, J. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 490.

†Since these experiments were completed we have carried out a series of similar studies using animals operated at 30 days of age. This latter work is sufficiently complete to indicate that the results obtained are similar to those reported above for adults, although the total fatalities are considerably greater.

Of 46 untreated controls only 2 (4.4%) survived for as long as 2 months and

Twelve out of 14 attempts to revive animals in the late stages of adrenal insufficiency, either by intraperitoneal normal saline injections or by feeding normal saline or the Rubin-Krick salt solution were unsuccessful. In 2 cases revival was effected. These revivals were not attempted until a fall in body temperature indicated severe adrenal insufficiency, although the animals could in all probability in every case have been revived with cortical extract.

7587 P

Improved Colorimetric Method for Determination of Bromide Concentration in Blood and Cerebrospinal Fluid.

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In our previous study[1] dealing with determinations of bromide in blood and in cerebrospinal fluid it was pointed out that with the colorimetric procedure of Hauptmann[2] one cannot recover the actual amount of bromide in blood serum. According to Wuth's assumption the precipitates of blood proteins retain a certain amount of bromide.[3] However, with another method[4] for the bromide determination in which, like Wuth, we also used protein-free filtrates, we were always able to recover the total amount of bromide dissolved in various specimens of blood serum.

Having inferred from these findings that proteins are not essentially responsible for the loss of bromide, we tried out *in vitro* the effect of various blood components on the bromide determination: Dissolving, respectively, uric acid, creatinine, urea, glucose, amino-acids (glycine), lactic acid, potassium sulfate, sodium carbonate, magnesium-ammonium phosphate, potassium iodide and sodium

these when killed for autopsy showed large accessories. Forty-four (95.6%) died within 34 days; the average survival was 8 days.

In 41 animals given salt treatment for 30 days after operation, only 18 (43.9%) died during the course of treatment, the average survival being 9 days. Twenty-three animals (56.1%) were alive when the treatment was discontinued. At this writing the survival of this group, after treatment was stopped, has not been determined.

1 Katzenelbogen, S., and Goldsmith, H., *Am. J. Psych.*, 1931, **10**, 1045.

2 Hauptmann, A., *Klin. Wochnschr.*, 1925, **4**, 1629.

3 Wuth, O., *J. A. M. A.*, 1927, **88**, 2013.

4 Hastings, A. B., and van Dyke, H. B., *J. Biol. Chem.*, 1931, **92**, 24.

chloride in aqueous solutions of sodium bromide, we found that only sodium chloride influenced the results obtained with Hauptmann's colorimetric determination of bromide: The more sodium chloride added, the lighter became the color, and the less bromide was found.

On the basis of this observation we attempted to modify Hauptmann's procedure, so as to eliminate the error arising from the presence of sodium chloride. The principle of our modification consists in equalizing (as nearly as possible) the NaCl concentration in the blood serum, spinal fluid and standard before carrying out the color reaction (due to formation of gold bromide). For that purpose we used, instead of distilled water, salt solutions for the dilution of the blood serum and for the preparation of the standard. In view of the fact that the cerebrospinal fluid commonly contains considerably less bromide than the blood serum, it is used undiluted for the bromide analysis. No sodium chloride need be added to the cerebrospinal fluid because its sodium chloride concentration very nearly approaches that of our blood serum dilutions and of the standards prepared with salt solutions.

TABLE I.

Experiments with serum			Experiments with spinal fluid		
NaBr added	(1) NaBr recovered	(2) NaBr recovered	NaBr added	(1) NaBr recovered	(2) NaBr recovered
50	42.0 (—16.0)	50.5 (+1.0)	10	8.6 (—14.0)	10.2 (+2.0)
100	84.3 (—15.7)	100.0 (0)	20	17.2 (—14.0)	20.3 (+1.5)
150	126.7 (—15.7)	149.0 (—0.7)	40	33.3 (—16.7)	40.0 (0)
200	165.7 (—17.1)	197.5 (—1.3)	60	48.3 (—19.5)	58.3 (—2.8)
250	203 (—17.2)	246 (—1.6)	80	60.0 (—25.0)	80.7 (+0.9)
300	245 (—18.3)	297 (—1.0)	100	69.0 (—31.0)	103.5 (+3.5)

(1) by Hauptmann's procedure, (2) by the modified procedure.
Figures are in mg. %. Figures in ( ) are differences in %.

Table 1 shows that with the original Hauptmann's procedure losses of bromide were registered; conversely, with our modified procedure the amounts of sodium bromide dissolved in various specimens of blood serum and of cerebrospinal fluid were totally recovered.

The technique of the modified method is essentially the same as the technique previously described,[1] except for the following differences: (a) Dilution of blood serum with a 0.75% NaCl solution (instead of water.) (b) The stock solution contains NaBr 166.8 mg.% (instead of 142 mg.%) in a 0.70% NaCl solution (instead of water). (c) The working standards are prepared with a 0.70% NaCl solution (instead of water).

7588 P

Acid Fuchsin for Demonstration of Ingestion in Paramecium caudatum.

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The study of ingestion in *Paramecium* is ordinarily accompanied by considerable difficulty due to the fact that there is little difference in color between the animal, the surrounding medium, and the bacteria which serve as food. The following method differentiates these elements by means of contrasting colors.

The dye used in this investigation was acid fuchsin, certification number NR-2, having a dye content of 62%. Solutions were prepared with distilled water so that their described percentages represented the actual dye strength.

Two drops of culture fluid containing large numbers of paramecia were placed on a clean slide, a drop of 1% solution of acid fuchsin added, and a cover glass applied. The fluid formed a thick layer under the cover glass, but the dye solution was unequally distributed. The preparation was allowed to stand undisturbed for one or 2 minutes, then examined under low magnification with reduced light. The paramecia, under these conditions, appeared to be outlined sharply in black. A portion of the fluid was then drawn off by the application of a strip of filter paper, thus reducing the thickness of the film of solution under the cover glass. In this thinner layer paramecia appeared to be colored a pale, luminous green, the color being deeper in those animals which had spent some time in the more deeply colored portions of the preparation before swimming out into the lighter areas. It must be emphasized here that this color phenomenon appears only when the amount of liquid under the cover glass is small; it will not appear if the cover glass "floats high."

If a quiet *Paramecium* is studied under high magnification, the process of ingestion and the formation of food vacuoles may be seen with a diagrammatic clearness. The green animal lies in a red or pink environment. Bacteria, stained pink or red, are swept into the cytopharynx which, due to the color of its fluid contents, appears a delicate pink. The undulating membrane can be clearly seen in action. At the tip of the cytopharynx the forming food vacuole may be observed, its contents in rapid motion due to the continuous entry of additional water and food. The pink vacuole is sharply set

off from the surrounding green protoplasm. Finally the sharp contraction of the protoplasm at the tip of the cytopharynx may be observed, and the course of the newly formed food vacuole, with its pink and red contents, followed in its passage through the cytoplasm.

It should be pointed out that this color phenomenon is in no way due to changes in pH. It is not, therefore, an indicator effect such as was described by Nirenstein,[1] The effect has been demonstrated in several microscopic organisms by the use of acid fuchsin, phenosafranin or erythrosin. It appears to be a physical phenomenon, closely related to the existence of extremely thin films of dye solution. Further study of this effect is under way.

7589 C

Polarization Studies in Tissue Models.*

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It is known that the electric resistance of animal tissues, contrary to that of solutions, is not determined by the law of Ohm alone.[1] This has been explained as consequence of polarization caused by semipermeable cell membranes at cell interfaces. The degree of polarization and, therefore, of the permeability can be measured by the capacitance or inductance needed to obtain a sharp minimum on the Wheatstone bridge. As further manifestation of polarization, the resistance of tissues decreases with the increasing frequency of the alternating current. While other authors worked mostly with methods based on the first phenomena, we used the difference in conductivity at high and low frequencies as measure of membrane polarization and permeability. In order to elucidate to some degree the chemical and colloid-chemical conditions underlying the polarization and permeability phenomena, different artificial membranes with various constituents were used.

A Wheatstone bridge was employed. Alternating currents of

[1] Nirenstein, Edmund, *Z. f. wiss. Zool.*, 1925, **125**, 513.

* Aided by a grant from the Ella Sachs-Plotz Foundation.

[1] Gildemeister, M., *Handb. d. norm. und pathol. Physiol.*, 1928, **8**, 657.

various frequencies (560 to 6890 cycles) were provided by an oscillator built according to the description of Jones and Joseph,[2] in combination with an amplifier.† The difference between the conductance at highest and lowest frequency was expressed in percentage of the conductivity at low frequency. This value will be called Δ in this paper. In order to obtain a sharp minimum, variable capacities (up to 0.5 M. F.) were employed. The conductance vessel consisted of 3 glass cells, the outer cells contained electrodes of platinum wire gauze covered with black of platinum. Their surface was 3.8 cm.², the distance was 21 mm. Between the outer cells and the middle cell one or 2 membranes could be placed. The apparatus was filled with KCl solutions, whose concentration varied depending on the resistance of the membrane. (.01-1 n solutions). The method was first checked on frog's skins which gave a Δ of 23% (high frequency 6890 cycles, low frequency 560 cycles).

Results. When parchment, collodium, 30% gelatine membranes hardened in formaldehyde (Collander[3]), 30% gelatine membranes containing 2.5% pseudoglobulin were measured, Δ did not exceed 0.4%. Thus proteins alone seem to play no or only a minimal part in the mechanism of polarization observed in tissues.

The behavior of lipoid membranes (containing egg lecithin Merck, pure lecithin, or kephalin from human brains) depends on their preparation. The first group of these membranes was prepared from collodium-ether solutions containing from .2-5% of the respective lipoids in molecular or lowly polymerized dispersion. These membranes showed definite polarization phenomena. (See Table I.)

In the preparation of the second group of the lipoid membranes, gelatine, up to a concentration of 30%, was added to watery colloid solutions of lecithin or kephalin. While the lecithin-gelatin membranes were translucent, the kephalin-gelatin membranes were

TABLE I.

Lecithin in %	Frequency of the alternating current low	high	Δ for 1 membrane	Δ for 2 membranes
.2	1115	4860	4.7%	
2.5	560	4860	9 %	40.6%
5	560	6890		71.2%
5	560	4860	15.7%	
5	560	6890	16.5%	

[2] Jones, G., and Joseph, R. C., *J. Am. Chem. Soc.*, 1928, **50**, 1049.

† We wish to express our thanks to Dr. G. Henny for his help in building the oscillator.

[3] Collander, R., *Protoplasma*, 1927, **3**, 213.

opaque. Nevertheless they gave the same results in the conductance measurements, both showing low values of Δ (maximum: .4%.)

These experiments show that polarization phenomena as observed in animal tissues can be imitated by lipoids. They show, furthermore, that the polarization, as measured by Δ, depends on the degree of dispersion of the lipoid in the membrane. Membranes containing the lipoid in fine dispersion show high degree of polarization (high value of Δ) as do animal tissues, while membranes with lipoids in coarse dispersion show no or only minimal polarization.

This conclusion is corroborated by microscopical studies of the lipoid membranes. The collodium-lecithin membranes are homogeneous, while the gelatin-lecithin membranes show doubly refracting lecithin lumps in irregular distribution.

The polarization of the lipoid membranes (as expressed by Δ) was increased when the membranes were placed in .05 n HCl for 24 hours. It was diminished or destroyed by analogous treatment with .05 n NaOH or 95% alcohol. The change in the alcohol was irreversible due to the extraction of lecithin. The polarization of lecithin membranes treated with alkali could be restored by subsequent exposure to acid (.05 n HCl).

These experiments on membranes were followed by similar studies on brain tissue. According to Nernst[4] and others, excitation is supposed to be due to a change of ion concentration on semipermeable membranes. Thus it seemed of interest to study agents that influence the convulsant reactivity in regard to their effect upon the polarization of brain tissue with this method. It was found on the cerebral hemispheres of cats and guinea pigs that Δ diminishes under the influence of agents that produce a swelling of the tissue (hypotonic salt solutions, alkali). These studies are being continued with special reference to convulsive reactions.

7590 P

Cause of Laxative Effect of Feeding Bran Pentosan and Cellulose to Man.

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Recent work suggests that the laxative effect of bran in animals and man is due to its fibre content. But in addition to fibre there

[4] Nernst, W., *Pflügers Arch. f. Physiol.*, 1908, **122**, 275.

are large amounts of pentosan in bran. The purpose of this work was to compare the laxative properties of these 2 nondigestible carbohydrates, and also to determine whether the laxative effect was due entirely to the physical property of increasing the amount of unabsorbable matter and water content of the stool, or whether the metabolic products formed by the action of intestinal bacteria on them might be stimulating to the intestine. The volatile fatty acids are one of the principal split products of the action of bacteria on both digestible and nondigestible carbohydrates. We have already shown them to be greatly increased when assimilable carbohydrate predominates in the diet.[1]

The plan of the work was to use 2 human subjects whose colons appeared normal by X-ray studies. Four feeding periods each of 7 days. The first period consisted of feeding a nonresidue basal diet; in the second period the basal diet was supplemented with 35 gm. daily of a crude preparation of pentosan from bran which contained 40% pentosan by weight; in the third period the basal diet was supplemented with 35 gm. daily of a fraction of bran containing 50% crude fibre; and in the fourth period apples, apricots, and prunes were added to the basal diet.

The stools were weighed immediately and steam sterilized and sealed in fruit jars. They were analysed for pentose by determining the nonfermentable sugar after acid hydrolysis and precipitated by mercuric sulphate. The fibre was determined by the usual Weender method as outlined in official methods of Association of Agricultural Chemists. Volatile acids by our own method.[2]

Of the pentosan fed, only 17% in one subject and 6.5% in the other was recovered in the stools. In contrast to these results, 102% and 95% of the fibre fed was recovered. The marked destruction of pentosan did not increase either the volume of the stools or the output of volatile fatty acids. When, however, fibre was fed, the volume of the stools increased in one subject 100% and in the other 88%. The volatile fatty acids also increased 56% in one subject and 74% in the other. During the period when fruits were added to the basal diet there were definite increases in the stool volumes and amounts of volatile fatty acids.

Conclusions. The pentosans of bran are not laxative while bran fibre is definitely so. The volatile fatty acids parallel the volume of the stool and are not increased by the breakdown of pentosan.

1 Grove, E. W., Olmsted, W. H., Koenig, Karl, *J. Biol. Chem.*, 1929, **85**, 127.

2 Olmsted, W. H., Duden, C. W., Whitaker, W. M., Parker, R. F., *J. Biol. Chem.*, 1929, **85**, 115.

7591 C

Effect of Gelatine Feeding upon Cases of Pseudohypertrophic Progressive Muscular Dystrophy.*

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The beneficial effects of glycine feeding upon several cases of muscular dystrophy reported by Milhorat, Techner and Thomas[1] prompted the writers to investigate the effect of prolonged gelatine feeding upon 3 boys,[2] well advanced cases of pseudohypertrophic progressive muscular dystrophy, patients in the Shriners' Hospital for Crippled Children.† The boys, C.E., M.S., and D.G. were 11, 9 and 8 years of age, respectively. D.G. was able to walk but could not arise from a sitting position; C.E. and M.S. were unable to walk and could move only by sitting and pushing themselves along with their hands. The boys were removed from bed each morning and encouraged to exercise as much as they would during the day. The period of study extended throughout most of one year and included a preliminary control period on a meat-free diet, a prolonged period of gelatine feeding without meat, followed by a period without gelatine. Creatinine and creatine determinations were run on carefully collected daily urine samples. At intervals the ability of the 3 children to exercise was determined by having them walk (or slide if unable to walk) until exhausted, a record being kept of the distance covered. A tabulation of data on C.E., the most severe case, is given in Table 1 as illustrative of the findings in all these cases. Gelatine feeding markedly increased the excretion of creatine in all cases as previously found by Gibson and Martin.[3] The increase was greatest in C.E., the most severe case, and least in D.G., the mildest case. An increase followed by a decrease was observed as found by Milhorat, Techner and Thomas during glycine

*The writers are indebted to the Charles B. Knox Gelatine Company, who kindly supplied the gelatine for the investigation.

1 Milhorat, A. T., Techner, F., and Thomas, K., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 609.

2 Freiberg, I. K., and West, E. S., *J. Biol. Chem.*, 1933, **101**, 449.

† These cases were used by Freiberg and West in a study of glycine synthesis under benzoate stimulation. The writers are indebted to Dr. C. H. Crego, Jr., and his staff of the Shriners' Hospital for Crippled Children in St. Louis for making these cases available, and for excellent coöperation in the study.

3 Gibson, R. B., and Martin, T. F., *J. Biol. Chem.*, 1921, **49**, 319.

feeding. The creatine excretion on a meat-free and gelatine-free diet after the period of gelatine feeding was essentially as in the control period before gelatine feeding. The creatinine coefficients before and after feeding gelatine were for C.E., 3.7 and 1.76‡; for M.S., 2.9 and 1.66; and for D.G., 3.5 and 2.6 respectively, representing a definite decrease in each case. Harris and Brand[4] have pointed out the correlation of a low creatinine coefficient and the severity of the disease. We have observed the same thing in 3 other cases (boys in the same family), in which the creatinine coefficients were inversely proportional to the severity of the condition.

TABLE I.

C. E., male, age 11 years.* Could not walk or rise from sitting position. Muscular weakness from infancy progressively becoming worse.

Periods days	Diet	Creatinine Mg. 24 hr. Aver.	Creatine Mg. 24 hr. Aver.	Exercise‡ Slid Feet	Exercise‡ in min.
24†	No meat, no gel.	247	400	182	15
24	" " 18 gm. gel.	233	527	161	20
13	" " 28 " "	243	790	364	25
16	" " 42 " "	214	808	298	15
3	" " 28 " "	246	692	1456	100
7	" " no gel.	247	641	1820	107
18	" " 28 gm. gel.	243	698	1456	135
14	Same	230	672	364	50
13	"	237	713	1456	142
17	"	191	660	546	110
17	No meat, no gel.	157	467	1638	150
24	Same	172	401	1350	175
20	Hospital diet	214	508	1456	200
12	No meat, no gel.	193	459	455	120

*Weight increased progressively from 25 kilos on 7-12-32 to 36 kilos on 3-30-33. Much of the increase was due to fat.

†Began 7-13-32. Periods continuous until 2-10-32, with the exception of a day or two between some of the periods. 20 days elapsed between periods 11 and 12, but the diet was unchanged. 2½ months elapsed between periods 13 and 14.

‡Exercise tests were given at intervals of 3 to 4 weeks, and included the interval between 9-22-32 and 10-11-33.

The last column of Table 1 gives the results of exercise tests on C.E., throughout and following gelatine feeding. Such tests have serious disadvantages, yet probably indicate something as to relative muscular ability. As judged by this test all showed considerable improvement during the gelatine feeding and 2§ maintained it for sometime after gelatine was discontinued.

Clinical examination of the muscles throughout the period of

‡ He had become quite fat during the experiment.

[4] Harris, M. M., and Brand, E., *J. A. M. A.*, 1933, **101**, 1047.

§ D. G. became unable to walk during April of 1933. Gelatine feeding was discontinued 1-24-33 in all cases.

observation showed little change with possibly some further degeneration. The creatine and creatinine excretions also indicate that the condition did not improve and probably became worse. Notwithstanding these facts, the general condition of the patients appeared some better during the gelatine feeding.

Fifteen gm. of glycine were fed daily beginning 7-16-'33, and continuing for 3 months in the case of M.S., 2 months in the case of D.G. and 2½ months in the case of C.E. The characteristic increase in creatine excretion was observed, the increase being little greater than caused by 28 gm. of gelatine (equivalent to about 7 gm. of glycine). This suggests that constituents in gelatine other than glycine caused considerable of the increased creatine excretion. The glycine feeding had no apparent beneficial effect.

The peculiar muscular sensations noted by Milhorat, Techner and Thomas during glycine feeding were not observed at any time in our cases.

The administration of ephedrine sulfate (4 doses of 3/8 grain at 3 hour intervals) to the cases while on a meat-free diet did not significantly change the creatine or creatinine excretion. Reinhold and others[5] reported the creatine excretion of a dystrophy case (type of dystrophy uncertain) as doubled by ephedrine administration while receiving glycine.

D.G. excreted 72% of 1.0 gm. of creatine when ingested after breakfast on a meat-free diet. C.E. and M.S. excreted 87 and 65% respectively.

Conclusions. Cases of pseudohypertrophic progressive muscular dystrophy seemed to be somewhat improved, as judged by exercise tests, by prolonged gelatine feeding. The improvement, if any, was of a temporary nature and the progress of the condition was not arrested. The creatinine coefficients were lower at the end of the gelatine feeding than before and the creatine excretion showed little change. We believe that gelatine feeding affords as much stimulation as glycine feeding in this condition.

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5 Reinhold, J. G., Clark, J. H., Kingsley, G. R., Custer, R. P., and McConnell, J. W., *J. A. M. A.*, 1934, **102**, 261.

7592 C

Possible Relation of Blood Groups to Age and Longevity.

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The writer's purpose is to point out hitherto unrecognized possibilities of blood-group investigations in relation to the problems of human constitution.

Landsteiner found that blood-group formation is physiological, independent of pathological processes.[1] This discovery and the findings of von Dungern and Hirszfeld[2] that groups A and B are transmitted as *dominants,* stimulated much additional research on the problems of serology, heredity, anthropology, paternity and constitution. Hirszfeld[3] has stressed the importance of further research on the relation of blood groups to the problems of human constitution.

The problems of constitution deal primarily with those innate characters which largely preserve individual identity; therefore only those characters which are classifiable into discernible types in the living, and which, after type differentiation, remain permanent, or relatively so, throughout the life span, may be useful in these problems. The results of all investigations show that blood groups are innate and indicate that, after type differentiation early in life, the group inherited by the person remains permanent, regardless of disease and other environmental influences;[4, 3] hence further research on the relation of blood groups to problems of human constitution may be of great promise, although the recorded results, thus far, are very contradictory. May not the reasons for such results be found in the lack of uniformity in technical details, race, stock or even community differences, inadequate numbers and controls and *possibly in the fact that blood groups have not yet been studied in relation to age and longevity?*

The relation of heredity to longevity has long been recognized. Genealogical and other studies show that longevity is heritable not only in man, but in lower forms.[5] In summing up his mathematical

[1] Landsteiner, K., *Wien klin. Woch. Bd.,* 1901, **14**, 1132.

[2] v. Dungern, E., und Hirszfeld, L., *Z. f. Immunitäts.,* 1910, **6**, 284.

[3] Hirszfeld, L., *Ergeb. Hyg. Bakteriol.,* 1926, **8**, 366.

[4] Lattes, L., *Die Individualität des Blutes,* Trans. by Schiff, F., Berlin, 1925.

[5] Pearl, R., The Rate of Living, pp. 1-18, New York, 1928.

discussion on the relation of heredity to duration of life, Pearl[6] states, it "Indicates that from one-half to three-fourths of the death rate is selective in character, because that proportion is determined by hereditary factors. Just in proportion as heredity determines the death rate so is the mortality selective." Obviously the known relation of heredity to longevity, whatever its magnitude may be, has a definite place in the problems of human constitution. But before this relation can be useful in these problems, the types of inherited characters must be shown to remain permanent, or relatively so, after type differentiation, and their possible age incidences must be investigated. *The only investigations of inherited characters in relation to age and longevity, recorded in the literature, are those on human scapulae.*

A brief summary of the writer's studies based on human scapulae will indicate the possibilities of similar studies of other inherited characters and among these, blood groups. Incident to family studies in 1906, he was led to classify the scapulae of man and some other mammals into *convex* and *scaphoid* (straight, concave and mixed) *types.* Observing in 1907, that *convex* types predominate in the *old* and *scaphoid* types in the young, he was led to investigate the origin, permanence, distribution and age incidence of scapular types, as well as their possible relation to longevity.[7–20] His investigations show: that, in man, the range in variation of scapular types is from the extremely *convex* through the *straight* to the extremely *concave;* their origin is primal; in man, they are transmitted with unusual constancy, regardless of sex, *the scaphoid* (straight, concave and mixed) *types* as *dominants;* they are differentiated in man in prenatal life; they remain *permanent* in type in man throughout the life span, regardless of ageing processes, nutrition, health, disease, oc-

[6] Pearl, R., The Biology of Death, p. 177, Philadelphia and London, 1922.

[7] Graves, W. W., *Med. Record* (N. Y.), 1910, **78**, 861.

[8] Graves, W. W., *Trans. Nat. Assn. for Study of Epilepsy and Care and Treatment of Epileptics,* 1911, **8**, 56.

[9] Graves, W .W., *Contrib. Med. and Biol. Research,* dedicated to Sir William Osler, 1919, **1**, 525.

[10] Graves, W. W., *Am. J. Physiol. Anthropol.,* 1921, **4**, 111.

[11] Graves, W. W., *Am. J. Phys. Anthrop.,* 1922, **5**, 21.

[12] Graves, W. W., Trans. Am. Assn. of Life Ins. Med. Directors, 1923. (Discussion: Dublin, L. L., Rogers, O. H., Patton, J. A., Hoffman, F. L., and Graves, W. W.)

[13] Graves, W. W., *Arch. Int. Med.,* 1924, **34**, 1.

[14] Graves, W. W., *Z. f. Konstitutions.,* 1925, **11**, 717.

[15] Graves, W. W., *Arch. Int. Med.,* 1925, **36**, 51.

[16] Graves, W. W., *Glasgow Med. J.,* 1925, 315.

cupation and other environmental influences; they are found in varying percentages in the remains of ancient and modern man and of some other mammals (gorilla, orang, champanzee, armadillo, bat *et al*); they are present in varying percentages in the excellently, well and poorly adaptable, regardless of age, race, stock and body build; in similar age periods, they may be present in varying percentages in different communities of the same race or stock; and in skeletal material and in healthy and sick groups, representing successive age periods from childhood to old age, the percentages of *convex* types *increase,* while those of *scaphoid* types decrease. His more recent figures on the age incidence of scapular types in white stocks are approximately as follows: 6 to 15 years: scaphoid types 65%, *convex* types 35%; 60 years and over: *scaphoid* types 35%, *convex* 65%.

Since adequate investigations, including follow-up, lead to the conclusion that scapular types remain *permanent* throughout the life span, the only tenable explanation for their age incidence is *better adaptability, less morbidity, greater longevity among the bearers of the convex than among the bearers of the scaphoid types.* This explanation is supported by the writer's figures and those of others[17, 19, 20] showing that in the healthy and sick groups studied in similar age periods there are approximately from 1¼ to 2¼ times as many *convex* types in the healthy as in the sick groups. Whether the types of other inherited characters will show similar age incidences in relation to the problems of constitution (adaptability, morbidity and longevity) cannot be known until such types have been similarly investigated.

Since blood groups had not been investigated in relation to age and longevity, and since it seemed that investigations of their possible age incidence might reveal the presence or absence of such relation, investigations were begun in 1933 by Mr. H. C. Pulley, Assistant, Department of Bacteriology and Dr. J. B. Mitchell, Jr., Instructor, Department of Pharmacology, St. Louis University School

[17] Graves, W. W., The Relations of Shoulder Blade Types to Problems of Mental and Physical Adaptability, The Henderson Trust Lecture, No. IV, Edinburgh, 1925.

[18] Graves, W. W., *Eugenics Rev.*, 1931, **23**, 215.

[19] Graves, W. W., A Note on Inherited Variations and Fitness Problems. I. The Types of Scapulae. Trans. Third Internat'l Congress of Eugenics, Baltimore, 1934.

[20] Graves, W. W., The Relation of Inherited Variations of Structure and Function to Problems of Health, Disease, Education, Duration of Life, and Adaptability in General. I. The Types of Scapulae. (In publication.)

of Medicine and Sister Mary Francis, Technician, University Hospital. The investigations of Sister Mary Francis deal with healthy and with hospitalized and out-patient material (white males and females) in successive age periods from birth onward. The results of her investigations will be published elsewhere. Those of Pulley and Mitchell are with white male students in the Medical School and ambulatory white male inmates of the St. Louis Infirmary (Alms House). The results of their investigations thus far available, arranged according to the international classification, are shown in Table I.

TABLE I.

Age	Total	O		A		B		AB	
yrs.		No.	%	No.	%	No.	%	No.	%
22-26	281	115	40.9	112	39.9	38	13.5	16	5.7
60+	500	232	46.4	203	40.6	48	9.6	17	3.4
		% increase 13.9		% increase 1.7		% decrease 28.8		% decrease 40.4	

As far as known, the first investigations on the possible age incidence of blood groups are those of Pulley and Mitchell and of Sister Mary Francis. Pulley's and Mitchell's results, thus far, are based on admittedly small numbers and while the number and percentage differences in relation to age are comparable, they are not conclusive, even for the age periods represented. However, the known age incidence of scapular types and its explanation suggest the possibilities of further blood-group investigations in relation to age. Such investigations by many workers with large numbers, representing healthy and sick groups of different races or stocks and different communities of the same race or stock, may disclose definite answers to the questions: Is there an age incidence of blood groups? If so, can it, like that of scapular types, be explained on the known relation of heredity to longevity? Affirmative answers to these questions will enlarge the usefulness of blood groups in relation to the problems of human constitution, as these are expressed in innate predisposition to health or disease, innate capacities for living and adaptability in general.

Acknowledgements. The writer wishes to thank Mr. H. C. Pulley and Dr. J. B. Mitchell, Jr., for the use of their figures and to express his appreciation of the encouragement given him by Professors Moyer S. Fleisher and John Auer in the effort to stimulate further research on the relation of blood groups to the problems of human constitution.

7593

Effect of Oestrin Injections upon Experimental Pancreatic Diabetes in the Monkey.*

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The anterior hypophysis has been shown to be related to carbohydrate metabolism by the studies of Houssay and Biasotti[1] in which the acute diabetes induced by pancreatectomy, in the dog, was relieved by removal of the anterior hypophysis. In a study based on the recognized action of oestrin in suppressing the anterior lobe it was shown by one of us[2] that administration of oestrin in depancreatized dogs had much the same effect as removal of the hypophysis.

Repetition and extension of this work on a form more closely related to man was believed to be of some importance. Accordingly we have employed the rhesus monkey in a series of experiments designed not only to repeat the studies on the dog, but also to gather data concerning the physiological mechanisms involved, and to determine the effect of continued oestrin administration, in depancreatized animals, upon certain other organs. At this time we are reporting the results of experiments conducted on 6 immature female monkeys (3 to 3.5 kg. in weight).

In 2 animals the injection of an acid extract of beef hypophyses[2] has induced hyperglycemia and glycosuria. We have used, uniformly, the Shaffer-Somogyi[3] method for sugar determination and have determined the true sugar as well as total reducing substances in both blood and urine. Twenty-four hour collections of urine were made regularly.

Four animals have been depancreatized. In 2 cases a small fragment of pancreas was not removed. In the remaining 2 animals pancreatectomy is believed to have been complete.

Two animals were treated with 100 R. U. oestrin (Theelin†)

* This investigation was aided by a grant from the Committee on Scientific Research of the American Medical Association to Dr. M. D. Overholser.

[1] Houssay, B. A., and Biasotti, A., *Endocrinology*, 1931, **15**, 511.

[2] Barnes, B. O., Reagan, J. F., and Nelson, W. O., *J. Am. Med. Assn.*, 1933, **101**, 926.

[3] Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 695.

† The pituitary extract was supplied through the kindness of Dr. Oliver Kamm and Dr. D. A. McGinty of Parke, Davis and Company. The Theelin used in this experiment was an oil solution, also supplied by Dr. Kamm.

daily for 2 weeks previous to and for 6 days following pancreatectomy. There was no appreciable glycosúria (.3 to .5 gm.) during the period following operation. However, when oestrin injections were suspended the urine sugar increased to 11 gm. When oestrin injections were resumed the urine sugar gradually decreased. In one animal the administration of 200 R. U. daily resulted in the total disappearance of sugar. Two animals received no oestrin prior to operation and were allowed to develop a glycosuria before injections were initiated. The injection of 100 R. U. daily resulted in the complete disappearance of sugar. Alternate periods of injections and withdrawal of injections resulted in corresponding fluctuations in the urine sugar. During one of the periods of treatment the animals also received injections of the pituitary extract. As a result sugar reappeared in the urine and persisted for several days. At the present time, over 5 months after operation, 3 of the 4 operated animals are alive and in good condition. The weight lost during periods when no oestrin was given has been more than compensated for under oestrin treatment. One monkey died 72 days after pancreatectomy. Her diabetes had been well-controlled according to blood and urine sugar findings. Autopsy showed the probable cause of death to be numerous metastatic abscesses in the liver and lungs from a pyogenic blood stream infection originating in an abdominal abscess present prior to operation. This abscess failed to respond to treatment and probably was chiefly responsible for the animal's death.

Blood sugar tests on normal fasting monkeys have shown a considerable range (55 to 125 mg. per 100 cc.). Following pancreatectomy it has risen as high as 550 mg. per 100 cc. and has been regularly decreased by the administration of oestrin. However, in the 2 totally pancreatectomized animals we have been unable to lower the blood sugar level to the normal level. For example monkey 17 had a normal level of 125 mg., a diabetic level of 550 mg., and a level after oestrin treatment of 185 mg.

It is believed that the evidence presented here may be interpreted as evidence that oestrin has suppresssd the diabetogenic activity of the anterior lobe. These studies are being continued and extended.

7594 C

On the Mechanism of Spastic Vascular Disease.*

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Two opposing views exist as to the mechanism of spastic disease of blood vessels. Some regard it as an expression of dysfunction of the vaso-motor nerve supply to the vessels. Chief support for this conception is found in the symmetrical nature of the lesion and the paroxysms which characterize it. Others, especially Lewis and his coworkers, regard it as a local fault not primarily associated with abnormal innervation. This local defect as studied in Raynaud's disease of extremities expresses itself in an abnormal response to cold, in the spatial order of development and disappearance of the vascular constriction, and in the failure of local anesthetization of the nerve supply to prevent or release completely an attack. Our own observations on these aspects of the disease lead us to support the contention of Lewis and others.

Evidence is herein presented that the fault is a local one, and represents not a hyperfunction of a sympathetic innervation, but a change in the blood vessels, namely that they respond to epinephrine in a manner similar to tissues deprived of their sympathetic nerve supply (paradoxical response) while the nerve supply can be demonstrated to be functional.

The evidence is derived from the study of 3 patients with vascular abnormalities of the upper extremities, 2 cases of Raynaud's disease and one case of acro-asphyxia. The first 2 were subjected to the following tests: (1) 1 cc. or less (graded doses) of epinephrine hydrochloride (1-1000) was given hypodermically and the effect on the diseased and control extremities noted. An attack was invariably induced in the diseased extremities. Then the effect of intravenous glucose or of a meal rich in carbohydrate was noted. (2) Ten to 15 units of insulin were administered to produce a physiological secretion of epinephrine. The effect on the diseased and control extremities was noted and again the effect of carbohydrate on the attack determined.

Case I. Male, age 37 with well advanced Raynaud's disease of fingers of both hands, the tip of one finger was gangrenous. Doses

* Assisted by a grant in Aid of Research in Neurophysiology from the Rockefeller Foundation.

of epinephrine, 0.2 cc. to 1 cc., hypodermically administered produced violent attacks with cyanosis, blanching, and pain. Attacks were relieved in 5 minutes by intravenous glucose. Fifteen units of insulin produced a severe attack in 50 minutes. The blood sugar dropped from 70 to 33 mg. %. Intravenous glucose quickly relieved attack. No effect occurred in vessels of feet or of other parts not obviously abnormal during either of the tests.

Case II. Male with well developed Raynaud's disease of upper extremities, gangrene of tips of 3 fingers on each hand. Response to epinephrine and insulin similar to that in Case I. Cyanosis after insulin extended almost to shoulders. The blood sugar dropped from 54 mg. % to 27 mg. %.

Case III. Male with definitely established attacks of hypoglycemia in which blood sugar dropped to 14 mg. %. During each attack patient's fingers blanched and caused him to experience burning pain. Removal of ¾ of the pancreas with relief from attacks of hypoglycemia also resulted in relief from attacks of white fingers.

These 3 cases demonstrated a local hypersensitiveness of the blood vessels to circulating epinephrine. There was normal or increased sweating and no loss of pilomotor function in the diseased extremities. No operation on sympathetic nerves had been carried out in any of these patients. Previously such hypersensitiveness to epinephrine on the part of smooth musculature has been noted following denervation. Investigation as to the nature of changes in tissues responding paradoxically to epinephrine is, therefore, being continued, particularly from the point of view of nervous function.

That the nerve supply to blood vessels was functional in these cases was demonstrated as follows: Anesthesia of the nerves of the arm in Case I resulted in definite dilatation of the vessels and cooling curves taken in a water-jacketed plethysmograph indicated greater constriction (faster cooling) in the hand normally than after anesthetization. Case II developed pain in all the fingers of one hand in a plethysmograph when the air was 21°C., although a thermometer between 2 fingers gangrenous at their tips read 34°C. This occurred within 5 minutes after cooling started, the air in the room being 32°C., and the initial temperature between the fingers 33.5°C. Reflex constrictions resulting in pain, therefore, occurred before significant cooling. Dipping the elbow of either arm in water at 6°C. resulted in pain in the fingers within 2 minutes homolaterally, but not contralaterally (local reflexes) with no measurable change in temperature of the palm or fingers. This pain persisted for 10 minutes with the arm in the air at 32°C., with cyanosis. The

patient was put in a cold room at 6°C. with both arms bundled to the shoulder in 3 inches of cotton batting. Pains occurred while the temperature between the fingers was still rising (initial T. 33.5° C., pain at 34°C., intense pain at 33.8°C.) and on coming out of the cold room the pain stopped in 4 minutes with no detectable change of temperature in the fingers of the wrapped hand (33.8°C.). The pain was obviously due to reflex spastic constrictions or circulating epinephrine and not to changes in temperature of the fingers. The alternative to the defect being a local one in the arteries would seem to be to suppose a hyperfunction of the nervous supply to these parts. This would not explain a paradoxical reaction to epinephrine.

These conditions could be explained by inferring a local (paradoxical) reaction to epinephrine or similarly acting body substances, but without the loss of nervous function which is the one known cause of such a condition. But the local reaction (spasm, pain) is not due only to circulating epinephrine; it can be called forth reflexly and also by local cooling of the anesthetized parts. That is, the affected parts go into spasm due to all the stimuli that would normally cause mild constriction, and pain and gangrene result secondarily from arterial spasm rather than from cold.

These findings may be applied to a better understanding of spastic vascular disease, such as Raynaud's disease of the extremities or certain cases of angina pectoris, etc., where external and internal alterations in environment are known to result in an increase in circulating constricting agents and also to produce 'attacks'. They cast doubt on the soundness of conception of surgical procedures in which the sympathetic nerve supply to blood vessels is interrupted. Procedures involving the removal of post-ganglionic neurones would tend especially to exaggerate to maximum degree an already existing fault. Procedures resulting in the interruption of pre-ganglionic neurones would exaggerate the fault to a lesser degree because the fully developed paradoxical response to epinephrine occurs only after removal of post-ganglionic neurones. Such procedures, while resulting in hyperaemia and in the relief of pain, may do so only temporarily until the aggravation of the condition due to denervation leaves the remedy more embarrassing than the original disease.

Uncomplicated arteriosclerosis to the extent of producing gangrene of the extremities does not involve anything resembling the paradoxical epinephrine reaction.

7595 P

Ovarian Irradiation and Sexual Precocity in the Rat.

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Incidental to some work with X-rays the writers have had occasion to carry out a series of experiments to determine the effects of X-ray treatment upon the time of sexual maturity in the rat as evidenced by the criteria of vaginal canalization and appearance of an oestrous smear.

Immature females, 7 to 10 days of age, were subjected to varying dosages of X-rays. One week later the same dosage in each case was repeated. The treatments were given under the following conditions: 200 k.v.p. (meter reading); 3 milliamps; 25 cm. target-to-specimen distance; 1 mm. Cu. filter. This resulted in a dosage of 41.4 roentgen units per minute as determined by a standard ionization chamber. The effective wave length, as found by the method of relative penetration of Al and Cu, was .13 Å.U. Dosages ranged from 540 to 1,240 'r' units; amounts above the latter figure proved fatal, death occurring within from a few days to as long as several weeks after the first irradiation.

Striking results were obtained in all the irradiated animals in that, regardless of dosage within the limits indicated, the vaginae opened at approximately 14 days after the first irradiation, at an average litter age of 22 days. Vaginal smears in each instance revealed a characteristic oestrous picture a day or two later, and the uterus, in each instance in which examination was made, appeared markedly distended. Twenty-three animals, representing 11 litters, showed this response. Controls, at least 1 litter mate for each experimental animal, in each case displayed vaginal opening followed by oestrus at 43-60 days—at least 3 weeks later than the irradiated animals.

The X-rayed ovaries of animals sacrificed at time of vaginal opening, 22 days of age approximately, revealed the presence of huge follicles, with enormous antra. Few follicles of small size were to be found. The ovaries of litter mate controls presented a marked contrast in appearance in that only small and medium-sized follicles were in evidence. It appears that the X-ray treatment had stimulated the growth of follicles tremendously; and that these follicles have been functionally hyperactive as well is evidenced by the fact that vaginal canalization was markedly precocious.

7596 C

Studies on the Neutral Sulfur of Urine. Criticism of the Iodimetric Titration of Diethylsulfide.*

GRACE MEDES, KIVELY EVANGELIDES AND KAMENOSUKE SHINOHARA.†

Abel[1] found diethylsulfide in dogs' urine which had been treated with calcium hydroxide. Christomanos[2] determined the amount of the sulfide in urine by iodimetry, basing his method upon the formation of the addition product of diethylsulfide and iodine, $(C_2H_5)_2SI_2$, previously reported by Cahours.[3]

TABLE I.

Iodine taken up by Et_2S from I_2 solutions, with variation in the different components of the system.

Conc. KI mols per l. × 10^2	Conc. I_2 mols per l. × 10^2	Conc. HI mols per l. × 10^2	Conc. H ions* mols per l. × 10^2	Et_2S Mols × 10^3 added to 1 l. sol.	I_2 Mols × 10^3 taken up	Ratio $\frac{I_2}{Et_2S}$
2.4	1.0			1.46	3.09	2.16
6.0	1.0			0.96	1.22	1.27
18.0	1.0			1.34	0.25	0.86
24.0	1.0			1.27	0.21	0.17
6.0	0.265			1.32	0.15	0.11
6.0	0.505			1.00	0.35	0.35
6.0	0.790			0.92	0.72	0.70
6.0	1.00			0.96	1.32	1.27
6.0	2.61			1.23	3.13	2.56
6.0	2.0			6.66	10.55	1.59
6.0	2.0			16.48	17.48	1.06
6.0	2.0			20.52	18.28	0.89
6.0	2.0			24.22	18.89	0.78
6.0	2.0			35.58	19.45	0.55
	2.0	8.0		1.83	2.80	1.53
	2.0	8.0		6.55	9.47	1.46
	2.0	8.0		17.95	16.77	0.93
	2.0	8.0		24.30	18.42	0.76
	2.0	8.0		34.61	19.00	0.55
	2.0		8.0	1.85	2.80	1.53
	2.0		6.0	1.81	2.76	1.52
	2.0		5.0	2.03	2.95	1.45
	2.0		3.0	2.27	3.22	1.42
	2.0		1.0	2.00	2.75	1.38
	2.0		0.0	1.80	2.29	1.27

*Hydrogen ion concentration was calculated by assuming the hydrogen iodide to be totally ionized. 0.04*N* iodine solutions in 0.08*M* KI and 0.08*M* HI were combined in various ratios so that the total concentration of electrolyte might remain constant.

*Aided by a grant from the Leffman Fund of the Wagner Free Institute of Science, Philadelphia.

† Robert McNeill Fellow of the McNeill Laboratories.

1 Abel, J. J., *Z. physiol. Chem.*, 1896, **20**, 253.

2 Christomanos, A. A., *Z. physiol. Chem.*, 1933, **217**, 177.

3 Cahours, *Ann. d. chim. et d. phys.*, 1865, **135**, 355.

The authors have been unable to confirm this stoichiometric relationship under the conditions described by Christomanos and investigated the factors influencing the distribution of iodine in the system, diethylsulfide—aqueous solutions of potassium iodide.

The experiments were carried out as follows: The diethylsulfide was weighed by difference from a weighing burette into 500 cc. volumetric flasks embedded in an ice-water mixture ($t = 2°$ to $5°$) and containing iodine, potassium iodide and hydrogen iodide. The mixtures were shaken until equilibrium was reached with respect to the iodine distribution. A portion of the mixture was centrifuged at 2° to 5°, and 25 cc. of the clear aqueous solution were subjected to iodimetric titration. The results are shown in Table I.

Results. The amount of iodine taken up by diethylsulfide varies with the amount of the sulfide and also with the concentration of iodine, iodide ion and hydrogen ion, the effect of the last factor being the slightest. This suggests that the removal of iodine from its solution by diethylsulfide is due to the solubility of iodine in the sulfide. In fact, iodine was found to be miscible with diethylsulfide roughly in all proportions.

In the light of the present experimental results the method for the determination of diethylsulfide in biological solutions should, therefore, be based upon other principles.

Summary. The amount of iodine taken up at a constant temperature by diethylsulfide is the function of the concentrations of iodine, iodide ion and hydrogen ion in addition to the amount of diethylsulfide added, and there is no such chemical relation under the experimental conditions as expressed by the equation:

$$(C_2H_5)_2S + I_2 = (C_2H_5)_2SI_2$$

The analytical method based upon this erroneous principle is unreliable.

7597 C

Effects of Avian Pituitary Glands in Salamanders.

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Induction of ovulation has been secured in various forms by administration of implants or extracts of the pituitary glands of

species and of classes other than that of the host animal.[1-7] Response of the host was negative, however, in the following cases: pigeon implants in mice,[1] implants of hypophyses of cow, dog, guinea-pig, rat, frog, fish, chicken, and snake under the skin of toads,[3] frog implants and mammalian implants and extracts in toads,[7] frog hypophyses in mice,[8] and in toads,[9] rat implants in toads.[10] Further experiments indicated that the probable explanation of these negative results, at least in some cases, was failure to administer the glands in adequate dosages. Thus by increasing the daily dosage Lipschütz, Kallas and Wilckens[11] were able to induce ovulation in mice with pigeon pituitaries, and Wills, Riley and Stubbs[10, 12] secured positive results in toads with frog and fish glands. The latter authors therefore concluded that, contrary to the conception of Houssay *et al.*,[3] there was no specificity of the maturity hormone of the anterior lobe of the pituitary among anurans. Their subsequent failure to obtain ovulation in toads by rat implants caused them to admit a possible specificity between these classes, with the alternative that the hormone might be destroyed due to the incompatibility of the tissues and subsequent reactions.[10] The following experiments, in which avian anterior lobes were administered to urodele hosts, do not support the idea of specificity of hormones and are reported as an additional case of induction of ovulation by heteroplastic implants and injections of pituitary. They are also concerned with the difference in potency between glands of young and adult donors, and with the effect of implants and injections on the thyroid of the host animal.

Implants of Adult Fowl Pituitaries. Glands were obtained from fowl (chiefly Rhode Island Red roosters) killed for market. The

1 Smith, P. E., and Engle, E. T., *Am. J. Anat.*, 1927, **40**, 159.

2 Zondek, B., und Aschheim, S., *Arch. f. Gynäkol.*, 1927, **130**, 1.

3 Houssay, B. A., Giusti, L., et Lascano-Gonzalez, J. M., *Compt. Rend. Soc. Biol.*, 1929, **102**, 864.

4 Adams, A. E., *Proc. Sec. Int. Cong. for Sex Res.*, 1930, 190.

5 Kehl, R., *Compt. Rend. Soc. Biol.*, 1930, **103**, 744.

6 Adams, A. E., *Anat. Rec.*, 1931, **49**, 37.

7 Adams, A. E., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 677.

8 Lipschütz, A., and Paëz, R., *Compt. Rend. Soc. Biol.*, 1928, **99**, 693.

9 Bardeen, H. W., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 846.

10 Wills, I. A., Riley, G. M., and Stubbs, E. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 784.

11 Lipschütz, A., Kallas, H., and Wilckens, E., *Comp. Rend. Soc. Biol.*, 1929, **100**, 28.

12 Wills, I. A., Riley, G. M., and Stubbs, E. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 411.

heads were kept on ice until needed when the pituitaries were removed and implanted in the back muscle of *Triturus viridescens* females. Implants were made from the same series of heads over a period of one week. Three animals were used as hosts and each received a definite third of the anterior lobe of each pituitary (Table I). Since 2 implants were made twice a day, each host received the equivalent in amount of approximately 1⅓ glands daily.

TABLE I.
Adult Fowl Anterior Lobes in Triturus Females.

Animal	Daily Dosage	Total Implants	Duration	No. of Eggs
CC1	2(2 × ⅓) glands	Ant. ⅓s = 8⅔ glands	6½ days	None
CC2	"	Mid. ⅓s = 6⅔ "	5 "	10 laid
CC3	"	Post. ⅓s = 8⅔ "	6½ "	7 in oviducts

No eggs were ovulated by control animals kept under the same conditions while one of the 3 experimental animals laid eggs and a second had eggs in the oviduct when killed on the eighth day. The failure of CC1 to ovulate is not necessarily due to the region of the gland implanted but is more likely an individual difference in sensitivity to the hormone.

Injections of Powdered Fowl Pituitaries. The anterior lobes of 35 pituitary glands from heads of Rhode Island Red roosters were treated with several changes of acetone, ground in a mortar, and the dry powder taken up in 0.6% salt solution. Injections of ½ to 1 cc. were made daily into each of 2 female Triturus from October 18th to 23rd inclusive. One of the host animals laid eggs after 2, the other after 5 injections. No estimate can be made of the glandular equivalent of the injected material as the larger particles would not pass through the injecting needle and were allowed to settle out. At least 31 eggs were ovulated by one female and at least 32 by the other before the termination of the experiment on the sixth day. As ovulation did not occur in control animals during this period, it may be concluded that the anterior lobe of the fowl pituitary possesses gonad-stimulating hormone, the potency of which is not destroyed by acetone.

An attempt was made to extract fowl anterior lobes in pyridine following drying in acetone, but the potency of the material was apparently destroyed at some point in the procedure as no eggs were ovulated by either of 2 females given 11 injections from October 25th to November 5th inclusive.

Implants of Chick Pituitaries. Rhode Island Red chicks, varying in age from one to 10 days, were decapitated and the anterior lobes of the pituitaries removed and implanted into the back muscle of 11

TABLE II.
Chick Anterior Lobes in Triturus Females.

Animal	Daily Dosage	Total Implants	Duration	No. of eggs
CH1	½ gland	7⅔ glands	22 days	None
CH2	,,	7½ ,,	22 ,,	,,
CH3	,,	12½ ,,	32 ,,	2 in ovid.
CH4	,,	11½ ,,	32 ,,	None
CH5	,,	11 ,,	32 ,,	,,
CH6	,,	11½ ,,	32 ,,	,,
CH7	2 × ½ glands	44 ,,	23 ,,	,,
CH8	,,	44 ,,	23 ,,	,,
CH9	2 × ½ (22 days) 2 × 1 (3d), 2 (3d) 2 × 2 (4d)	50 ,,	53 ,,	6 laid 7 in ovid.
CH10	2 × ½ (22d) 2 × 1 (3d), 2 (5d) 2 × 2 (8d) 2 × 3 (8d)	118 ,,	70 ,,	1 laid 3 in ovid.
Hypo'sectomized	2 × ½ (2d) 2 × 1 (4d), 2 (2d)	14 ,,	14 ,,	14 in ovid.

Triturus viridescens females (Table II). In 4 cases ovulation occurred, while none of a control series ovulated. The fact that 3 of the 4 pituitary-treated newts that ovulated received at least 2 glands per day at some time during the experiment, while only one (CH3) out of 8 receiving daily from one-half to one gland ovulated, seems to indicate that the latter may have been too small a daily dosage to cause ovulation except in very sensitive host animals. This is in line with the results, mentioned earlier in this paper, obtained after implants of pigeon pituitaries in mice and of frog pituitaries in toads, where a relatively small daily dosage gave negative, a larger, positive results. The need for the larger amount of chick pituitary might result from destruction of hormone by the host, failure of the tissue to release the hormone, or, and this seems more likely in the light of the results from adult glands, to the slight gonad-stimulating potency in the glands of young donors.

As a check, a series of 11 hosts were given implants of Triturus pituitaries and in every case ovulation occurred after 3 to 15 implants (average 7.5). Thus both the *total amount* and the *amount per dose* of Triturus gland tissue required for ovulation in Triturus were less than the *amounts* of fresh chick or fresh fowl tissue required. The average of the total *number* of glands of Triturus and of adult fowl was almost exactly the same, (7.5 Triturus, 7.6 fowl), but this may be merely a coincidence. Certainly the number of animals concerned is too small to draw any conclusion on this point.

Effect of Avian Pituitaries on Thyroid of Triturus. The thyroids of control animals and of hosts receiving Triturus, chick, powdered fowl, and fresh fowl anterior lobes were fixed in picro-aceto-formol

and stained in iron hematoxylin and Mallory B, and compared as to degree of stimulation. The thyroids of animals with Triturus and with adult fowl implants presented a picture similar to that obtained in Triturus by Adams[13] following injection of phyone and hebin for short periods. The height of the epithelium was increased over that found in normal glands, there were chromophobe vacuoles bordering the colloid indicative, according to Severinghaus,[14] of colloid absorption, and droplets of colloid within the cells. Implants of chick pituitaries likewise caused stimulation of the thyroids, slightly greater after 7⅔ glands than that found in normal or muscle-injected controls, markedly greater after 118 glands and after injection of powdered fowl glands in 0.6% salt solution. After both of the latter types of treatment, the thyroid picture resembled that obtained by Adams[13] after long-continued injection of phyone and hebin, with hypertrophy and hyperplasia of the gland, high follicular epithelium, scanty colloid in the follicles, and increased vascularity. Practically all the glands possessed the type of active follicle described by Severinghaus[14] in the duck and characterized by high active epithelium on one side, and lower, inactive appearing cells on the other.

The fact that an hypophysectomized animal injected with chick anterior lobe molted after having received 14 glands also suggested that its thyroid was affected, since Adams *et al.*[15] have demonstrated that thyroidectomized animals will molt after thyroid but not after pituitary implants. Histological examination of the thyroid of this animal gave evidence of stimulation in that some of the epithelial cells were cuboidal rather than flat and apical vacuoles were present in the cells of some follicles, indicating colloid absorption. Such evidence was lacking in the thyroids of hypophysectomized controls.

Summary. Anterior lobes of pituitaries of adult fowl (as fresh implants or powdered and injected in salt solution), or of young chick pituitaries (if a sufficient number of glands is implanted daily), will cause ovulation out of season in *Triturus viridescens* females. The thyroids of these females are stimulated beyond the normal condition. These results indicate that no specificity of hormone or hormones in ovulation-inducing and thyroid-stimulating capacities exists between birds and amphibians.

13 Adams, A. E., *Anat. Rec.*, 1934, **59**, 349.

14 Severinghaus, A. E., *Z. f. Zell. u. mik. Anat.*, 1933, **19**, 653.

15 Adams, A. E., Kuder, A., and Richards, L., *J. Exp. Zool.*, 1932, **63**, 1.

7598 P

Further Studies on Mechanism of Invasiveness by Pyogenic Bacteria.

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The writer has recently demonstrated that the dissemination of a foreign substance from its site of inoculation is at least in part a function of its irritating capacity.[1, 2] *Staphylococcus aureus* induces a lesion sufficiently intense to occlude draining lymphatics and to cause the formation of a fibrinous network as early as one hour after cutaneous inoculation of the organism. The degree of "walling-off" was determined by studying the dissemination of trypan blue from the site of injury to the regional lymphatics. In the case of Type I *pneumococcus* the area is circumscribed at a somewhat later stage (about 6 hours subsequent to the inoculation of the organism). When *Streptococcus hemolyticus* is inoculated into the skin the lymphatics maintain their functional patency for about 2 days, and throughout that time these vessels are virtually unoccluded by thrombi. This histological observation adequately accounts for the delayed fixation and consequently the relative ease with which the dye penetrates to the regional lymphatics in a hemolytic streptococcal inflammation.

These results, while offering an explanation for the well-known localizing tendencies of the staphylococcus as against the disseminating properties of streptococcus, present an interesting paradox. Staphylococci tend to remain localized and produce relatively slight systemic effects because of their pronounced local injurious action which serve to fix them *in situ*. Hemolytic streptococci, on the contrary, produce far greater systemic sequelae owing to the invasiveness resulting from their relatively mild local effects.

The experiments of the writer have recently been confirmed by Dennis and Berberian.[3] Subsequent to the writer's observations, Tillett and Garner[4] have demonstrated that broth cultures of hemolytic streptococci are capable of liquefying the normal human fibrin clot. In contrast to this they pointed out that the normal rabbit fibrin clot is totally resistant to dissolution by such means. This

[1] Menkin, V., *J. Exp. Med.*, 1933, **57**, 977.

[2] Menkin, V., *Arch. Path.*, 1931, **12**, 802.

[3] Dennis, E. W., and Berberian, D. A., Proc. Soc. Exp. Biol. and Med., 1934, **31**, 976.

[4] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

finding renders it difficult at present to accept the opinion of some writers[3] that the *in vitro* lytic element observed by Tillett and Garner is an essential factor in accounting for the delayed fixation of a dye demonstrated by the author in the streptococcal type of inflammation in rabbits. It is possible that the fibrinolytic effect of hemolytic streptococci on the human plasma clot plays a definite rôle in human infections; but this is rather in the nature of a reinforcing or accessory factor added presumably to a more fundamental property of the hemolytic streptococcus which holds true for both the human and rodent types of infection.

In an attempt to analyze the problem further, experiments were performed with Berkefeld filtrates of the various types of pyogenic organisms previously studied. The details of this investigation will form the subject of a future communication, but the essential findings are summarized as follows:

1. The sterile filtrate of a several day old culture of *Staphylococcus aureus* induces an intense inflammatory reaction in the dermis of rabbits indistinguishable from the reaction obtained when the viable organisms are inoculated. Trypan blue is fixed as early as one hour after injection of the filtrate. Microscopic sections through the inflamed areas reveal many lymphatics occluded by a fibrinous reticulum. The tissue spaces are in many regions distended by coagulated plasma.

2. The staphylococcal filtrate is inactivated when heated for about one hour and a half at 58°C., for its cutaneous injection causes no fixation of the dye. Histologically, the lymphatic vessels are found to be entirely patent.

3. *Staphylococcus aureus* filtrate in contact with leucocytes obtained from an exudate causes these cells to become swollen, vacuolated, and, in many instances, degenerated. There is also some evidence that the total leucocyte count is lowered when this bacterial filtrate is maintained for some time in contact with an exudate. No such effect on leucocytes is produced with the heated and therefore inactivated staphylococcal filtrate. These observations strongly suggest that the active principle in the filtrate of *Staphylococcus aureus* which causes early blockage is somewhat similar, if perhaps not identical, to leucocidin.

4. On the other hand, the filtrates of both *Streptococcus hemolyticus* and Type I *pneumococcus* fail to induce retention of trypan blue at the site of cutaneous inoculation. There is no evidence of fixation even as late as 50 hours after skin injection of the streptococcal filtrate.

5. The strain of *Streptococcus hemolyticus* (S-23) used in all

these studies fails to inhibit the coagulation of rabbit blood; this holds true for the filtrate as well.

In conclusion, the foregoing preliminary data indicate that the localizing property of *Staphylococcus aureus* is probably referable not merely to the severe irritating property of the organism *per se,* but also to its additional ability to release a powerful soluble exotoxin-like product, identical in many respects with leucocidin, and capable in itself of inducing a sufficiently intense injury to cause obstruction of normal lymphatic drainage. *Pneumococcus* Type I and *Streptococcus hemolyticus,* on the other hand, fail to form any such detectable accessory substance able to cause damage to the lymphatic or capillary endothelium. Briefly, then, evidences obtained thus far are in accord with the writer's original view, that the delayed fixation of dye and hence the invasive capacity of hemolytic streptococcus is referable to the mild local effects of this organism in contrast to the pronounced injurious action of *Staphylococcus aureus.* These studies are being continued in an attempt to obtain more precise information concerning the chemistry and the rôle of leucocidin-like substances.

7599 P

Anticoagulants of the Blood.

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Any agent which inactivates or removes from blood, calcium, prothrombin, or thrombin is an anticoagulant. Theoretically, antifibrinogen and antithromboplastin are also possible but have never been convincingly demonstrated. The removal of calcium by oxalates, citrates, or fluorides is well known. As a type of antiprothrombin, aluminum hydroxide is an excellent example. On mixing and incubating oxalated plasma (0.5 cc.) with aluminum hydroxide cream (0.05 cc.) a plasma is obtained which after the removal of the aluminium hydroxide, will not clot on recalcification, whereas the untreated plasma clots in 2 minutes when calcium is added. The loss of clotting power is not due to removal of thromboplastin, for on adding an active preparation made from rabbit's brain, the normal clotting time is not restored. Furthermore, fibrinogen is not removed, for thrombin (fresh serum) will cause clot-

ting in 10 seconds. Prothrombin must be the constituent that is removed or inactivated by aluminum hydroxide. Significantly, this reagent does not remove or inactivate thrombin.

Hirudin is a type of true antithrombin. Heparin likewise appears to be an antithrombin as the following experiment demonstrates:

Fibrinogen 0.5% solution (cc.)	0.1	0.1	0.1	0.1	0.1
Fresh serum (cc.)	0.1	0.1	0.1	0.1	0.1
Heparin (mg.)	0.0	0.003	0.006	0.009	0.012
Clotting time (sec.)	15	60	300	No clot	

Heparin is not neutralized by thromboplastin. Even when excess thromboplastin is present, the clotting time is prolonged as the concentration of heparin is increased. Plasma containing 0.2 mg. of heparin per cc. will not clot, irrespective of the excess of thromboplastin.

Other substances, notably certain dyes, are antithrombic. Calcomine Fast Pink 2 B.L Unbl.* is a strong anticlotting agent.

Fibrinogen 0.5% solution (cc.)	0.1	0.1	0.1	0.1
Fresh serum (cc.)	0.1	0.1	0.1	0.1
Calcomine fast pink (mg.)	0.0	0.01	0.03	0.10
Clotting time (sec.)	10	15	30	300

There is no evidence that the presence of thrombin in the blood stimulates the production of antithrombin. On injecting 70 cc. of freshly defibrinated blood containing a high concentration of thrombin into dog (Body weight 13 kg.) no intravascular clotting occurred, but free thrombin was still present in the blood 40 minutes after the injection. On withdrawing 9 cc. of blood and mixing it with 1 cc. of M/10 sodium oxalate (an amount amply sufficient to prevent normal blood from clotting) a solid clot was formed in less than 24 hours by merely allowing the blood to stand. This must be attributed to the free thrombin still present.

* The dye, Calcomine Fast Pink 2 B.L. Unbl. was kindly furnished by The Calco Chemical Company.

7600 P

Anti-Serum Against Black Widow Spider Venom.

FRANCES E. BECKER AND FRED E. D'AMOUR. (Introduced by R. G. Gustavson.)

From the Research Laboratories, University of Denver, Colo.

The most important, if not the only poisonous spider found in the United States is the so-called Black Widow *(Latrodectus mactans),* which is widely distributed and by no means uncommon. Some experimental work concerning the properties of the venom have appeared but to our knowledge no one has presented clear-cut evidence of having obtained anti-serum of high potency. There has also been considerable doubt as to whether the venom is different from the arachnolysin present in all spiders and which reaches its highest concentration in the eggs.

The authors have studied the black widow spider from the standpoint of its natural history and the chemical, physiological, pharmacological and immunological properties of its venom. Approximately one thousand spiders and several hundred rats have been used. The results of this investigation will be published elsewhere, we wish to report here only on the questions raised in the preceding paragraph.

Much of the previous work published was done on the effects of the spider bite. This is open to the criticism that the amount of venom introduced would vary with the size and anger of the spider and the amount and speed of absorption would vary with the depth to which the fangs had penetrated. Our method was to dissect the pair of venom glands from each of a large number of spiders (20 to 100 were used in each batch), and macerate the glands in saline. Injections were made intraperitoneally. A toxicity curve was plotted, 10 rats being used for each dosage, and the average lethal dose determined. The eggs were macerated in saline and the average lethal dose determined. We found that one-fourth the venom in one spider would kill 5 rats out of 10, while one-half of the venom would kill 9 out of 10. For the eggs, one egg would kill 4 out of 10, while 2 eggs would kill 10 of 10 rats. For the venom, therefore, one-fourth spider is considered the average lethal dose, and for the eggs, one and one-fourth eggs is considered the average lethal dose.

A group of rats was injected every other day for 2 months with sub-lethal amounts of venom and another group with sub-lethal amounts of eggs for the same period. At the end of that time

the immunity and cross-immunity was tested. The results follow.

Immunity. 8 rats immunized with venom were given venom in double the amount necessary to kill control animals. No symptoms appeared and all animals survived.

Three rats were given 8 A.L.D. each of venom which had stood overnight mixed with 1 cc. of serum from immunized rats. No symptoms appeared and all animals recovered. Venom treated with normal serum was not altered in potency.

Four rats were given 8 A.L.D. each of venom followed immediately by 0.5 cc. of serum from immunized rats. No symptoms appeared and all animals recovered.

Four rats were given 8 A.L.D. of venom followed one hour later by 1 cc. of serum. Symptoms had already appeared. Recovery was prompt and all animals recovered completely.

Four rats immunized against eggs were given 4 A.L.D. of egg extract. No symptoms appeared. All survived.

Two rats were given 4 A.L.D. of eggs after standing overnight mixed with 1 cc. serum from egg-immunized rats. All survived without symptoms. Normal serum did not affect the potency of the egg poison, the rats dying within a few hours.

The above results represent the largest amounts of venom and egg poison used. Probably the potency of the serum is higher than indicated by these figures. This work is being continued.

Cross-Immunization. 3 rats given 2 A.L.D. of venom which had stood overnight with serum from egg-immunized rats died within 6 hours.

Three rats given 2 A.L.D. of eggs which had stood overnight mixed with serum-immunized rats died within 4 hours.

Immunity against eggs appeared more rapidly than against the venom. In the latter group little immunity could be demonstrated as the result of injections for the first month.

Summary. Anti-sera of considerable potency against both the venom and the eggs of *Latrodectus mactans* have been prepared. Cross-immunization experiments indicate that the 2 poisons are not identical.

7601 C

Carbohydrate Nature of Pantothenic Acid (Williams).

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Williams *et al.*[1] found that extracts from diverse tissues, representing different biological groups, contained material which had a very striking stimulation on the growth of Gebrüde Mayer yeast. From a similarity of biological and chemical reactions it was concluded that this stimulation was due to a single substance, which they called pantothenic acid, and which appeared "to have a molecular weight of about 150, to be distinctly acid without amphoteric properties, to have several hydroxyl groups in its structure, but no olefin double bond, aldehyde, ketone, sulfhydryl, basic nitrogen, aromatic or sugar groups."

Following the discovery of this acid, experiments were conducted in this laboratory to determine the effect, if any, of this substance upon bacterial growth. The organism which seemed most suitable for this work was *Escherichia coli,* since it was readily cultured and possessed considerable carbohydrate fermentative ability. Rice bran was employed because it was readily obtained and conveniently extracted.

Preliminary experiments with extracts of rice bran which were prepared by the method of Williams, refluxing the bran with a 60% methyl alcohol solution, etc., showed considerable stimulation of the organism with small amounts of the extract, but much less stimulation than observed by Williams with yeast. The results in many cases were inconsistent, due principally to the low pH of the extract and to the variations of the inoculum.

To overcome these inconsistencies and to standardize the procedure, all the extracts were first adjusted to a pH between 6.9 and 7.3. Inoculations were made with a constant amount of a broth suspension of the organisms, generally 0.2 cc. No means were attempted to standardize the infusion media, no 2 batches of which were the same.

In later experiments a different procedure was used for the extraction of the rice bran. This procedure, which was used for the experiment to be reported, was as follows: 800 grams of rice bran were mixed with 3 liters of 25% aqueous ethyl alcohol solution and

[1] Williams, R. J., Lyman, C. M., Goodyear, G. H., Truesdail, J. H., and Holaday, D., *J. Am. Chem. Soc.*, 1933, **55**, 2912.

allowed to stand, with frequent stirring, in an incubator at 40°C. for 48 hours. The liquid was then filtered off, the yield being about 1850 cc. This solution was then evaporated on a steam bath to about 250 cc., filtered, and the volume made up to 500 cc. A portion of this solution was then adjusted to pH 7.1 and allowed to set for 30 minutes in the ice box. A slight precipitate was observed. Filtration was made through a Seitz filter, while still cold, which removed the precipitate and rendered the solution sterile. This extract was then kept in the ice box until ready to be used.

In the data reported each growth determination was made in duplicate. Inoculations were made with 18-hour cultures of *Escherichia coli* or *Alcaligenes fecalis*. In every case 0.2 cc. of a 0.1% broth suspension was used. In addition to the various amounts of solutions reported in the tables, 5.0 cc. of infusion medium from the same batch were added to each tube, making the total volume in each case 12.2 cc. Growth was determined by centrifuging 10.0 cc. of each culture in a Hopkins tube for 1 hour at a standard speed, and recording the growth in cubic centimeters. All tubes, including the control tubes for the original pH, were incubated together at 37°C. for 8 hours. The hydrogen ion concentrations were determined with the hydrogen electrode. The buffer employed was McIlvaine's Na_2HPO_4—Citric acid[2] of pH 7.0. The glucose solution was prepared so that 1.0 cc. gave a 1% solution. The saline used was a 0.9% sodium chloride solution.

From the results of the extract in various dilutions upon the growth of *Escherichia coli* in unbuffered media (Table I) it will be

TABLE I.

Effect of Extract on *E. coli* in Unbuffered Medium.

Extract cc.	Saline cc.	Growth (cc.)			Gas	pH	
		1st tube	2nd tube	Aver.		Original	Final
0.0	7.0	.007	.007	.007	—	7.18	6.64
0.1	6.9	.012	.013	.012	+	7.19	6.09
0.5	6.5	.016	.017	.016	+	7.17	4.92
1.0	6.0	.023	.022	.022	+	7.16	4.82

observed that there is a steady increase in growth with increasing amounts of the extract, but that the maximum stimulation has not been reached with the amounts of extract used. On the other hand, with varying amounts of glucose, under like conditions, (Table II) there is a constant stimulation which does not increase with increasing amounts of glucose. The decrease in pH in the latter case

[2] Clark, W. M., "The determination of hydrogen ions." Williams and Wilkins, Baltimore, Md. 1928. p. 214.

is much greater than in the former, and is probably the limiting factor of the growth stimulation.

TABLE II.
Effect of Glucose on *E. coli* in Unbuffered Medium.

Glucose cc.	Saline cc.	Growth (cc.)			Gas	pH	
		1st tube	2nd tube	Aver.		Original	Final
0.0	7.0	.007	.007	.007	—	7.18	6.64
0.1	6.9	.013	.013	.013	+	7.18	5.71
0.3	6.7	.012	.013	.012	+	7.18	4.78
0.5	6.5	.013	.013	.013	+	7.18	4.74
1.0	6.0	.012	.013	.012	+	7.18	4.69

TABLE III.
Effect of Extract on *E. coli* in Buffered Medium.

Extract cc.	Buffer cc.	Saline cc.	Growth (cc.)			Gas	pH	
			1st tube	2nd tube	Aver.		Original	Final
0.0	6.0	1.0	.010	.008	.009	—	6.88	6.83
0.1	6.0	0.9	.016	.018	.017	—	6.87	6.70
0.5	6.0	0.5	.046	.048	.047	+	6.88	6.17
1.0	6.0	0.0	.058	.058	.058	+	6.88	5.69

TABLE IV.
Effect of Glucose on *E. coli* in Buffered Medium.

Glucose cc.	Buffer cc.	Saline cc.	Growth (cc.)			Gas	pH	
			1st tube	2nd tube	Aver.		Original	Final
0.0	6.0	1.0	.010	.008	.009	—	6.88	6.83
0.1	6.0	0.9	.022	.022	.022	—	6.87	6.63
0.3	6.0	0.7	.046	.044	.045	+	6.86	6.15
0.5	6.0	0.5	.045	.047	.046	+	6.87	5.62
1.0	6.0	0.0	.047	.048	.047	+	6.86	5.46

This is borne out more strikingly when buffered media were used (Tables III and IV), for with the extract much greater stimulation was recorded than in the unbuffered media, as was also true with glucose. However, the stimulation due to the glucose reaches a maximum, while that due to the extract does not. It is also observed that the stimulation produced by the extract is greater than that produced by the glucose and that there is a correspondingly smaller decrease in the pH with the former than with the latter, as compared with the growth increase.

When glucose is added together with the extract (Tables V and VI) there is no cumulative stimulation as would be expected if the stimulating agent were similar to glucose, nor is there a constant and unchanging stimulation as was observed with glucose alone (Table II). It will be noted that the final pH does not decrease with

TABLE V.
Effect of Extract on *E. coli* in Presence of 0.5% Glucose.

Extract cc.	Glucose cc.	Saline cc.	Growth (cc.) 1st tube	2nd tube	Aver.	Gas	pH Original	Final
0.0	0.5	6.5	.013	.013	.013	+	7.18	4.74
0.1	0.5	6.4	.013	.013	.013	+	7.19	4.77
0.5	0.5	6.0	.016	.018	.017	+	7.17	4.79
1.0	0.5	5.5	.020	.020	.020	+	7.16	4.85

TABLE VI.
Effect of Extract on *E. coli* in Presence of 1.0% Glucose.

Extract cc.	Glucose cc.	Saline cc.	Growth (cc.) 1st tube	2nd tube	Aver.	Gas	pH Original	Final
0.0	1.0	6.0	.012	.013	.012	+	7.18	4.69
0.1	1.0	5.9	.012	.013	.012	+	7.19	4.71
0.5	1.0	5.5	.016	.017	.016	+	7.17	4.76
1.0	1.0	5.0	.023	.023	.023	+	7.16	4.85

increasing amounts of the extract, but on the other hand, increases. The probable explanation of this is that the extract itself contains some buffering salts.

Gas was produced during growth in all cases except with the 0.1 cc. dilution of the extract in buffered medium (Table III) and the 0.1 cc. dilution of glucose under the same conditions (Table IV). In these cases it is probable that the carbonic acid formed was less than the amount required to neutralize the buffer, and with no excess, no gas bubbles were observed to rise and collect at the surface.

To show this more conclusively, another experiment was performed (Table VII). Five cc. of infusion medium were added to

TABLE VII.
The Final pH with Various Amounts of Extract and Buffer. Gas was produced in all tubes except those marked *.

Extract cc.	Buffer 2.0 cc.	4.0 cc.	6.0 cc.	8.0 cc.
0.1	6.28	6.40*	6.61*	6.69*
0.5	5.45	5.75	6.02	6.19
1.0	5.25	5.52	5.89	6.08
2.0	5.29	5.58	5.78	5.97

each tube as listed in Table VII, and the total volume made up to 15.0 cc. with saline. Inoculations were the same as in other experiments. Incubation time was 6 hours. These data show that with smaller amounts of the buffer, gas was observed in all cases where the final pH was less than 6.3, which coincides with the data shown in Tables III and IV.

The effect of the extract upon *Alcaligenes fecalis* is entirely negative. This organism is known to have no carbohydrate-fermenting ability. As would be expected, the pH increases rather than decreases.

From the facts that the extract causes a decrease in the final pH, the production of gas, and has no stimulating effect on the growth of *Alcaligenes fecalis,* the assumption can be made that the stimulating agent, probably pantothenic acid, is related to the carbohydrates. The stimulation observed with the extract, however, can in no way be attributed to glucose or other hexoses which stimulate *E. coli* in a manner similar to glucose.

Conclusion. 1. Rice bran contains a growth-stimulating agent for *Escherichia coli,* but not for *Alcaligenes fecalis.* 2. This stimulating agent is probably related to the sugars. 3. It is not a hexose. 4. The substance is probably identical with the pantothenic acid of Williams.

7602

Isolation of Glycocyamine from Urine.

C. J. WEBER. (Introduced by E. A. Doisy.)

From the Department of Internal Medicine, University of Kansas School of Medicine.

The isolation of glycocyamine (guanido acetic acid) from urine is of interest because of its possible relationship to the origin of creatine. Hunter has critically reviewed the literature on this subject in his monograph on "Creatine and Creatinine".

My interest in this subject began with the finding that an extract of urine prepared with Lloyd's reagent gave a positive Sakaguchi reaction. I also found that the Lloyd's extract of urine from a case of pseudohypertrophic muscular dystrophy gave a stronger Sakaguchi reaction than did urine from normal individuals. Urine from this patient (8-year-old boy) was therefore used in attempting to isolate the substance responsible for the positive reaction. The patient received 15 gm. of glycine daily during the period of urine collection. The urine was collected in 2- or 3-day periods using toluene as a preservative.

The procedure used is briefly as follows: The urine after filtering was made acid to congo red with hydrochloric acid and extracted

with 100 gm. Lloyd's reagent. The extractives were removed from the Lloyd's with barium hydroxide. The barium was removed from the filtrate with sulphuric acid. The filtrate containing creatine, creatinine and substances giving a positive Sakaguchi reaction was concentrated *in vacuo* to a volume of approximately 300 ml. This concentrated extract was kept in the refrigerator until there had accumulated a series of extracts from a total of 14.8 liters of urine representing a collection period of 18 days. These concentrates were then combined and evaporated to a volume of 1400 ml. and treated in the usual manner with phosphotungstic acid. Sixty per cent of the substances responsible for the positive Sakaguchi reaction remain in the phosphotungstic acid filtrate. The filtrate is freed of phosphotungstic acid with barium hydroxide and the excess barium removed with sulfuric acid. The filtrate is evaporated almost to dryness and extracted with absolute alcohol. The residue insoluble in alcohol was dissolved in water and sufficient sulphuric acid added to give a 2% concentration. This solution was autoclaved at 15 pounds pressure for 30 minutes and was then again treated with phosphotungstic acid. The filtrate was evaporated to 25 cc. The addition of picric acid to this concentrated filtrate resulted in the formation of a picrate which was recrystallized once from water. Six hundred and sixty mg. of the picrate was obtained which retained its crystalline form after drying at 100°C. for several hours. The picrate melted with decomposition at 200.6° and showed no depression of melting point when mixed with glycocyamine picrate. This picrate gave a positive Sakaguchi reaction and gave the same amount of color as glycocyamine picrate when equal quantities were compared.

Three hundred and fifty mg. of the picrate was suspended in 10 ml. of 20% sulfuric acid and extracted with benzene. The sulphuric acid was removed with barium hydroxide and the solution evaporated to a volume of 5 ml., from which, on cooling, crystals were obtained in the form of rhomboid plates. These were recrystallized once from water and dried at 90°C. Dr. S. A. Thayer of St. Louis University analyzed these crystals and obtained the following results:

3.813 mg. gave 2.02 mg. H_2O and 4.312 mg. CO_2
Glycocyamine, $C_3H_7O_2N_3$ (117) calculated C 30.77 H 5.98
found C 30.83 H 5.93
1.890 mg. gave 0.632 cc. N at 30° C., B.P. 734 at 0°
calculated N 35.87
found N 35.68

In another case of muscular dystrophy (12-year-old boy) receiv-

ing 15 gm. of glycine daily, 274 mg. of glycocyamine picrate was isolated from the urine over an 8-day period. In this case the urine was collected daily and extracted with Lloyd's reagent on the same day. The quantity of glycocyamine picrate isolated represents 23% of the original Sakaguchi reacting substances in the Lloyd's extract using glycocyamine as the standard for comparison.

In a control experiment 600 mg. of glycocyamine was added to a solution containing creatin, creatinine, glycine, urea and salts in concentrations approximating that found in the above 8-day urine collection. This mixture was treated by exactly the same procedure used above. Three hundred and ninety-six mg. of glycocyamine picrate was isolated which represents a recovery of approximately 33% of the glycocyamine extracted by Lloyd's reagent as determined by the Sakaguchi reaction. This suggests that probably the major part if not all of the Sakaguchi reacting substance in the Lloyd's extract from urine is glycocyamine.

Glycocyamine is not produced during the process of isolation, at least, from creatine, creatinine or glycine and urea. The addition of creatine, creatinine, glycine or urea together, or separately, to urine causes no increase in the Sakaguchi reaction of the Lloyd's extract.

The feeding of glycine to a patient with pseudohypertrophic muscular dystrophy results in an increase of approximately 60% in the Sakaguchi reacting substances in the urine.

7603 P

Relation of Cytoplasmic Structure to Growth and Respiration in Plasmodium.

A. R. MOORE.

From the Effingham B. Morris Biological Farm of the Wistar Institute of Anatomy and Biology.

Recently I have shown that if a small piece of the plasmodium of *Physarum polycephalum* be forced through a gauze with sufficiently small pores (less than 0.25 mm. in diameter) subsequent growth of this material does not occur, whereas if the pores are larger, growth takes place when the material is put on the culture medium. The experiments were repeated and confirmed for *P. rigidum.* During the present summer I have confirmed in the

main the previous results and have extended the experiments to follow the respiratory changes in *P. polycephalum*. As gauze sieves I have used Latimer's "Old Anchor Brand" calibrated bolting cloth Nos. 00, 4, 6, 8, 10, 14, and 21 which have average pore sizes of 0.75, 0.32, 0.24, 0.20, 0.155, 0.10 and 0.07 respectively. When strained through the last 3 the plasmodium gave no growth; when the 0.20 sieve was used the strained plasmodium sometimes grew, sometimes not; with sieves of larger pores active plasmodium when forced through, grew on the culture medium. On the other hand, plasmodium if allowed to grow in gauze bags, passed through all the sieves as well as hard paper filters onto the culture medium. In 5 hours plasmodium made its way through hard filter S. & S. No. 575A which has an average pore size of 1 μ. These facts confirm the conclusions previously stated that essential to the life of the plasmodium are filaments or fibrils which may be 0.20 mm. in length but in diameter are below visibility with the microscope.

Since viability is so conspicuously reduced by comminuting the plasmodium it seemed important to determine whether metabolism as indicated by consumption of oxygen might not also be modified in this way. The determinations were carried out in the usual way with the Warburg apparatus. A quantity of plasmodium, approximately 20 mg., was spread in a thin layer on a piece of platinum foil and introduced into the respiration chamber which was then charged with oxygen. The temperature of the bath was kept at 21° C. One or 2 normal controls were run with the experimental charges. Half hourly readings were taken over a period of 3 hours. The samples were then removed, dried to constant weight over soda lime, and weighed to the nearest 0.1 mg. The experiments all showed that O_2 consumption was reduced as a result of forcing the plasmodium through sieves, and that the O_2 consumed decreased with lessening of the pore size of the sieve used. The average value of O_2 consumption for normal plasmodium (36 readings) was found to be 4.3 cu. mm. per mg. dry weight per hour. For material passed through 0.75 mm. pores the O_2 consumption was 73% of normal, while through 0.07 mm. pores the figure was 49% of normal. Thus a degree of comminution which always killed the plasmodium reduced the consumption of O_2 by about 50%. These facts suggest that structures in the cytoplasm of *P. polycephalum* are essential both to its life and to the greater part of its respiratory activity.

It was further found that deformation of the plasmodium without comminution caused a delay in growth and a reduction in O_2

consumption. The deformation was accomplished by centrifuging the samples with an air driven centrifuge (H. W. Morse's model of the Henri-Huguenard centrifuge) at approximately 75,000 x gravity for from 1 to 10 minutes. Centrifuging for 6 minutes caused a delay of about 12 hours in subsequent growth of the piece, and an immediate reduction in the consumption of oxygen to near 50%. These results indicate that growth and respiration depend upon an intimate association of elements of relatively low and high specific gravity in the plasmodium, that these can be separated by centrifuging to the impairment of growth and respiration. But unlike comminution, the effects of separation by centrifuging are reversible.

7604 P

Influence of Closed Intestinal Loop Strangulation on Volume of the Combined Digestive Secretions.*

JOSEPH M. SWINDT AND M. LAURENCE MONTGOMERY. (Introduced by C. D. Leake.)

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We have reported our findings[1] on the influence of simple duodenal obstruction upon the volume of the combined gastric, biliary, pancreatic, and duodenal secretions. In the present work we have studied the influence of closed intestinal loop strangulation upon these same secretions. Twenty-two fasting healthy dogs were used in the study. All had their salivary ducts ligated.

In the first group of animals the duodenum was divided below the entrance of the lower pancreatic duct, a Dragstedt type of intestinal cannula was placed in the proximal duodenum and the cut ends were inverted. A second cannula was placed in the jejunum about 6 to 8 cm. distal to Treitz' ligament. From the duodenal cannula the combined digestive secretions drained into a clean rubber bag. Collections were made every one to 4 hours day and night, measured, and returned by cannula into the jejunum. About 50 cc. of Ringer's

*This work was conducted under a grant from the Christine Breon Fund for Medical Research of the University of California Medical School.

1 Montgomery, M. L., and Swindt, J. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 915.

solution or tap water were added alternately to the returned secretions. In addition to the fluids mentioned 500 to 1500 cc. of Ringer's solution were given subcutaneously daily to prevent dehydration. The control period lasted from 3 to 7 days. After the animal had fully recovered from the operation and the volume of the secretions had reached a constant level a second operation was performed. The jejunum was divided and the ends inverted just proximal to the jejunal cannula, producing an isolated duodeno-jejunal loop 15 to 20 cm. long around Treitz' ligament. Following this the collections and replacements were made in the usual manner. When replacement of the secretions was impossible larger amounts of Ringer's solution were given subcutaneously. In the obstructive period vomiting occurred. The volume of vomitus was prorated and added to the amounts of secretion collected. In 6 of the 10 animals the results were complicated by the following occurrences: gastric bleeding, perforation of the loop without distention, leakage around the cannula, failure of the secretions to assume a stable level during the control period, and internal hernia obstruction. In 5 of these animals the secretions were diminished in volume after the production of the isolated loop. In the animal with the bleeding into the stomach and duodenum the secretions were increased.

In the remaining 4 dogs the results were free from complications. Two of these were operated on under ether, and 2 under 2% novocaine anesthesia. Only one of the latter 2 had morphine prior to operation. In the control period the secretions reached a level in from one to 2 days averaging from 50 to 70 cc. per hour. Following the obstruction these animals survived for from 2 to 4 days during which time the secretions never exceeded 45 cc. per hour. In each individual instance the volume of the digestive secretions after the production of the isolated loop was definitely less than the volume obtained during the control period. At autopsy these 4 animals showed a distended gangrenous loop without gross perforation.

It was thought that the second operation might have a depressant influence upon the digestive organs. Therefore, in a second group of 12 dogs an effort was made to produce an isolated loop without the second operation. Various methods tried on 8 animals failed. Then in 4 dogs the duodenal and jejunal cannulae were placed as in the first group and an isolated duodeno-jejunal loop was made with a round flanged brass cannula placed by means of 2 purse string sutures into the distal end to drain the loop. During the control

period the collections and replacements of the digestive secretions were made in the usual manner. Then the isolated loop was closed by plugging the drainage cannula with cotton and screwing a cap on the end. In 2 of these animals there was leakage around the cannula which allowed the loop to decompress and prevented strangulation. However, in 2 animals the closure was perfect. The loops became distended, necrotic, and perforated as revealed at autopsy. During the control period the secretions in these 2 animals averaged about 65 cc. per hour, whereas the highest value after obstruction of the loop was 50 cc. per hour. Often it was much lower. One animal showed a sharp terminal rise in secretion, which, however, did not exceed the average for the control period. The character and amount of the immediately preceding secretions indicated this probably was due to terminal relaxation of the pylorus and emptying of accumulated fluid in the stomach rather than to stimulation. In the second dog the stomach was aspirated frequently during the period of obstruction and the terminal rise did not occur. It is our belief, therefore, that in isolated duodeno-jejunal loop strangulation obstruction the combined gastric, pancreatic, biliary and upper duodenal secretions are not stimulated to excessive secretion. On the contrary they tend to be depressed.

7605

Embolism by Air and Oxygen: Comparative Studies.*

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Attempts were made in these experiments to compare the minimal fatal dose of air and oxygen when introduced into a peripheral vein. Van Allen, Hrdina and Clarke[1] found that in embolism due to air introduced into the pulmonary vein, the rapidity of introduction of the air and the position of the animal were 2 factors of prime importance. The position of the animal was believed to be of importance by directing the flow of air upward by gravity. These authors found that when the head was uppermost, the air more easily pro-

* Work done in part under a grant from the Douglas Smith Foundation. Preliminary report.

[1] Van Allen, C. M., Hrdina, L. S., and Clarke, J., *Arch. Surg.*, 1929, **19**, 567

duced death. In embolism due to introduction of air into a peripheral vein, the air goes first to the heart and lungs independent of the position of the animal. In the present studies, all animals were kept flat in the supine position during the injection of gas. The gas was injected quite rapidly; in most instances the entire amount being introduced in about 30 seconds.

The minimal fatal dose of air was found to be about 8 cc. per kilo body weight. Animals under urethane anesthesia were used throughout the work. The air was injected through a large cannula into the femoral vein (usually the left) from an inverted burette, being forced in by a column of water. Another cannula was placed in the carotid artery to record the blood pressure. The air flowed in rapidly until at a certain point it began to flow more slowly and the column of water showed pulsations synchronous with the heart beat. This usually occurred when very roughly nine-tenths of the minimal fatal dose was injected. The results of 11 air injections are shown in Table 1.

TABLE I.
Effects of Injection of Air into the Femoral Vein.

No.	Dog Wt. Kg.	Cc. air injected	Cc. per Kg.	Time of injection sec.	Result
1	6.5	150	23.1	960	death
2	7.0	55	7.9	205	"
3	9.0	45	5.0	7	"
4	9.5	47	5.0	75	recovery
5a	8.0	54	6.8	10	"
5b	8.0	64	8.0	10	death
6	8.5	68	8.0	22	"
7	12.0	68	5.7	38	recovery
8	12.0	72	6.0	32	death
9	6.9	64	9.3	16	"
10	7.0	56	8.0	18	"
11	19.0	178	9.4	40	"

A dog that recovered following an injection of air or oxygen usually showed a rapid fall in blood pressure to as low as 20 mm. of mercury with gradual recovery to the normal level. There was usually no diminution in pulse pressure, but some slowing of the heart rate. Necropsy shortly after recovery in one dog showed no air or oxygen in the heart. Recovery was usually complete within 2 or 3 minutes.

A dog that died following an injection of air or oxygen usually showed a rapid fall in blood pressure to about 16 mm. of mercury with no return to the normal level. There was a rapid decrease in the pulse pressure with some slowing of the heart rate. The pulse

became imperceptible to palpation and on the blood pressure tracing. However, as long as 3 minutes after the last visible cardiac pulsations, a few abortive respirations would occur. In some cases a few weak cardiac pulsations occurred as long as 8 minutes after death as seen when the chest was opened. Necropsy showed air or oxygen in the vena cava and right side of the heart.

Oxygen injections were only slightly less toxic than air injections as seen in Table II. All effects on the blood pressure, respiration, and pulse were identical.

TABLE II.
Effects of Injection of Oxygen into the Femoral Vein.

No.	Dog Wt. Kg.	Cc. air injected	Cc. per Kg.	Time of injection sec.	Result
12a	7.8	62	8.0	20	recovery
12b	7.8	62	8.0	22	"
12c	7.8	69	8.9	26	"
12d	7.8	78	10.0	37	"
13a	9.5	65	6.8	30	"
13b	9.5	76	8.0	25	death
14	9.5	75	7.9	35	"
15	8.0	72	9.0	38	"
16	6.5	72	11.0	28	"
17	7.5	75	10.0	33	"
18a	8.0	80	10.0	30	recovery
18b	8.0	100	12.5	22	death

In an 8 kg. dog there is about 615 cc. of blood (assuming one-thirteenth of the body weight is blood). The arterial blood may be considered to be completely saturated with oxygen. Then assuming that half of the blood in the body is venous and that this half is 6% unsaturated with oxygen (it is realized that the unsaturation depends on vasomotor conditions and varies in various parts of the body), then the blood of the entire body of an 8 kg. dog is 0.06 x 308 = 19 cc. unsaturated. Since air is about 20% oxygen, and the minimal fatal dose of air for such a dog is 64 cc., this amount of air would contain 13 cc. oxygen. Thus the air would be taken up by the hemoglobin to the extent of 13 cc. while 19 cc. of oxygen might be taken up. Theoretically therefore, from the standpoint of combining with hemoglobin, the 2 should act practically the same. It is quite possible that because of the rapidity of the injection, the oxygen or air does not have time to mix with all the venous blood. Except for this it might be expected that an 8 kg. dog could tolerate 19 cc. more air or oxygen than pure nitrogen.

An attempt was made in experiments 13b and 15 to increase the oxygen unsaturation by clamping off the trachea for 60 and 75

seconds respectively just before injecting the oxygen. It is seen that the oxygen tolerance was not increased by this procedure. No blood studies were made in these dogs.

Calculations are made that indicate that the minimal fatal dose of oxygen should not be greater than that of air when injected rapidly into the peripheral vein of a dog. The results of 24 injections of air or oxygen into 18 dogs show no marked difference in toxicity.

7606 C

Studies on Centrifuged Frog Eggs.

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The effect of centrifuging fertilized eggs of the frog both before cleavage and during the early cleavage stages has been studied by several investigators. Gurwitsch,[1] Hertwig,[2] Wetzel,[3] Morgan,[4] Konopocka,[5] McClendon,[6] and Jenkinson[7] particularly have reported the redistribution of certain materials and the intense stratification of others along the primary axis of centrifuged frog eggs. They state that the stratification of materials takes place in the order of their relative specific gravity, *i. e.*, a yellow or white centripetal layer, a translucent protoplasmic middle layer and a heavy yolk centrifugal layer containing the black pigment granules. In general, the results of these investigators show that, when the centrifuging has been relatively weak, development is often apparently normal with perhaps a slight abnormal pigmentation and distribution of fat in the head region. However, if the centrifugal force has been slightly greater, curious monsters often appear. Jenkinson particularly has noted that the myotomes and spinal ganglia are frequently fused together beneath the nerve tube and that the notochord is often absent altogether. Hertwig frequently obtained monstrosities of the spina bifida type. Pasquini and Reverberi[8] have centri-

1 Gurwitsch, A., *Verhandl. Anat. Gesellsch.*, 1904, **18**, 146.
2 Hertwig, O., *Arch. f. Mikr. Anat.*, 1904, **63**, 643.
3 Wetzel, G., *Arch. f. Mikr. Anat.*, 1904, **63**, 636.
4 Morgan, T. H., *Arch. f. Entw.-Mech.*, 1906, **22**, 553.
5 Konopocka, B., *Bull. Int. Acad. Crac.*, 1909, Ser. B., 689.
6 McClendon, J. F., *Arch. f. Entw.-Mech.*, 1909, **27**, 247.
7 Jenkinson, J. W., *Quart. J. Micr. Sci.*, 1915, **60**, 61.
8 Pasquini, P., and Reverberi, G., *Boll. Inst. di Zool.*, Univ. Roma, 1929, **7**, 1.

fuged frog eggs at the gastrula stage and obtained many curious monsters not unlike some that have appeared in our experiments.

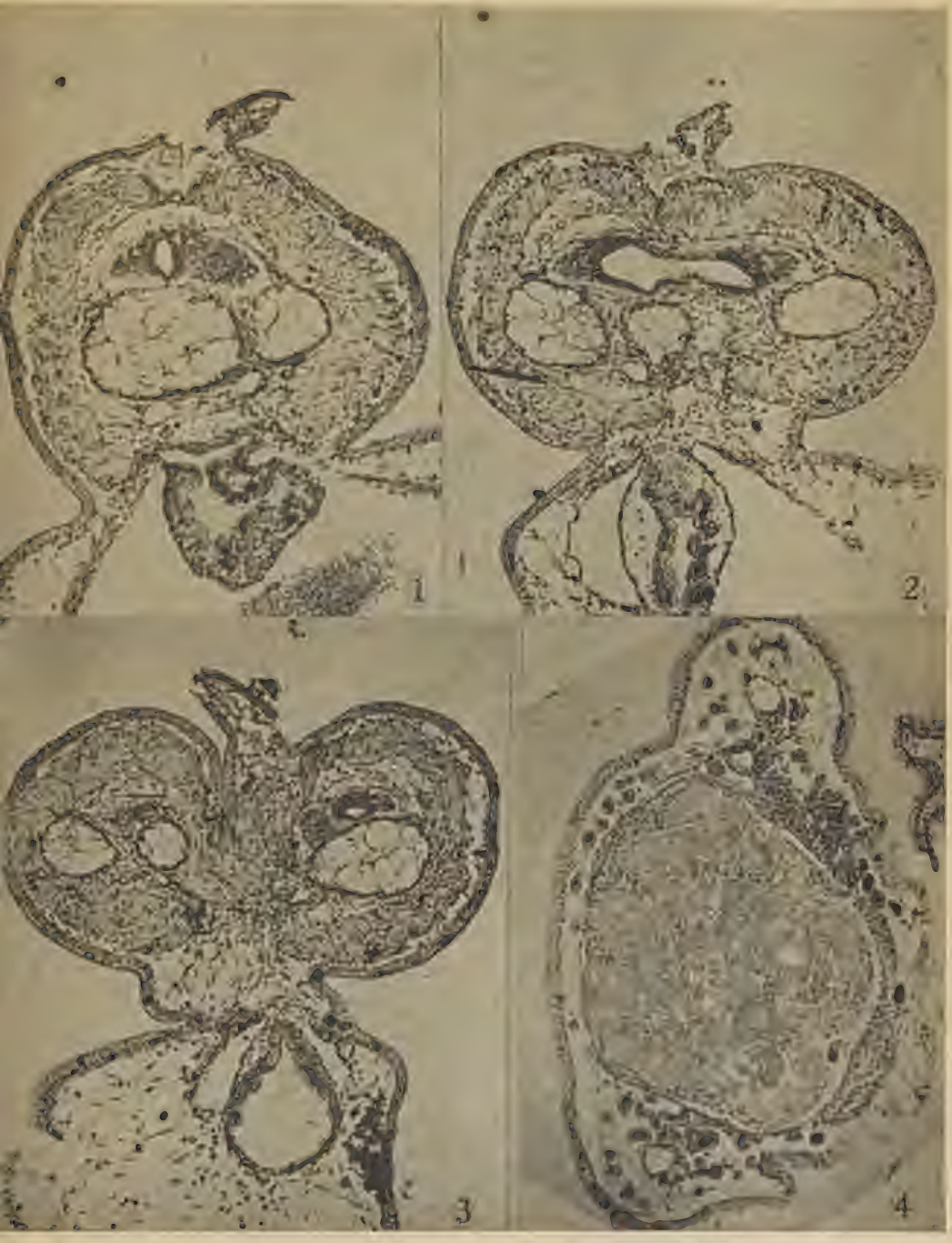
We have recently centrifuged unfertilized frog eggs of *Rana aurora* for 2 minutes at 40 pounds pressure in the air-driven ultracentrifuge described by Beams, Weed and Pickles.[9] It has been possible to repeat the stratification of materials in the frog egg as described by the above named investigators. Furthermore, we have produced a complete cleavage or fragmentation of the egg into a light fatty fragment and a heavier yolk and protoplasmic fragment. No effort has been made to determine whether or not such fragments may be fertilized and if fertilized will develop. When stronger centrifugal force is applied the fragments are completely forced out of the jelly and burst.

In another series of experiments the eggs of *Rana pipiens* in the many cell and early gastrula stages were centrifuged for 5 to 7 minutes at about 2000 times gravity. Such eggs often develop into monsters, many of which possess 2 and sometimes 3 tails. Figure 1 is a cross section through the region of the base of the tail of such a tadpole. It will be noted that the neural tube is single but shows some indication of spreading and becoming divided as in Figures 2 and 3 which are serial sections of the same tadpole taken slightly more caudad. The notochord has already doubled in Figure 1, and in Figures 2 and 3 the right notochord has divided so that there are 3 distinct notochords with 2 neural tubes. Sections taken cephalad show the neural tube and notochord to be single.

Figure 4 represents a cross section of another tadpole of a similarly treated group of eggs preserved at a slightly earlier period in development. There are two distinct notochords present but no trace of a neural tube. In this animal the brain showed distinct malformation and the neural tube extended posteriorly only for a very short distance. Other monstrosities with no heads and expanded growths of epidermis into folds and ridges were found. In still other cases the yolk was collected in the blastopore region forming an unusually large and persistent yolk plug which prevented the normal closure of the blastopore.

No special effort was made to orient the eggs in the centrifuge and it is assumed that they arranged themselves in the usual way with the vegetal pole outward or centrifugally. About one-third of the eggs which received the proper degree of centrifuging developed abnormally. Controls developed normally in every case.

[9] Beams, J. W., Weed, A. J., and Pickles, E. G., *Science*, 1933, **78**, 338.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We are unable to offer a definite explanation regarding the cause of the doubling of the neural tube and notochord and of the other curious malformations mentioned above. However, they may be due to mechanical disturbances in development caused by a displacement of mitotic figures, by the injury and possible killing of certain cells, or by the intense stratification of materials which results in an upset of the processes of development. On the other hand, it seems equally plausible from the work of Spemann and his collaborators that centrifuging in the many cell and early gastrula stages may have affected the composition or organization of the "inductors" or organizing centers resulting in a disturbance of the normal inductive process.

7607 C

Morphological Comparison of Anterior Pituitaries of Normal Castrated Female Rats and Those Receiving Injections of Pregnancy Urine Extracts.*

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It has been reported by Severinghaus[1] and Wolfe, Ellison and Rosenfeld[2, 3] that while injections of extracts of pregnancy urine result in marked changes in the anterior pituitaries of normal female rats, such extracts are without action on the anterior pituitaries of castrated female rats. In our previous reports[2, 3] detailed data were not given; in this report we wish to present quantitative data which demonstrate that the anterior pituitaries of non-injected castrated female rats (both mature and immature) are morphologically indistinguishable from those of female rats receiving injections of extracts of pregnancy urine.

Sixty-five virgin mature and 31 immature female rats were cas-

* These studies were supported by grants from the Committee for Research in Problems of Sex of the National Research Council, from the Committee for Scientific Research of the American Medical Association, and the Division of Medical Sciences of the Rockefeller Foundation.

1 Severinghaus, A. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 593.

2 Wolfe, J. M., Ellison, E. T., and Rosenfeld, Louis, *Anat. Rec.*, 1934, **58**, 93 (supplement).

3 Wolfe, J. M., Ellison, E. T., and Rosenfeld, Louis, *Anat. Rec.*, 1934, **58**, 94 (supplement).

TABLE I.

Period of castration	Percentage of Cells	Frequency Distribution				Mean and Standard Deviation (Percent			
		Controls		Experimentals		Controls		Experimentals	
		Mature	Immature	Mature	Immature	Mature	Immature	Mature	Immature
	Eosinophiles								
	30.0-34.9	2	2	4	2				
	35.0-39.9	8	10	7	11	Mean 36.5	Mean 38.4	Mean 37.5	Mean 38.3
	40.0-44.9	—	3	4	3	S. D. 2.0	S. D. 2.9	S. D. 3.6	S. D. 2.8
	Basophiles								
	5.0- 6.9	1	—	2	—				
15-day	7.0- 8.9	3	—	6	—				
	9.0-10.9	4	—	7	—	Mean 9.4	Mean 16.0	Mean 8.7	Mean 16.1
	11.0-12.9	2	1	—	—	S. D. 1.8	S. D. 2.4	S. D. 1.3	S. D. 1.4
	13.0-14.9	—	5	—	3				
	15.0-16.9	—	4	—	9				
	17.0-18.9	—	4	—	4				
	19.0-20.9	—	—	—	—				
	21.0-22.9	—	1	—	—				
	Chromophobes								
	35.0-39.9	—	—	—	—				
	40.0-44.9	—	5	—	4				
	45.0-49.9	—	9	2	11	Mean 55.0	Mean 46.1	Mean 53.8	Mean 46.5
	50.0-54.9	5	1	8	1	S. D. 2.5	S. D. 2.9	S. D. 5.2	S. D. 2.6
	55.0-59.9	5	—	4	—				
	60.0-64.9	—	—	1	—				
	Eosinophiles								
	35.0-39.9	2	—	15	—	Mean 43.0	—	Mean 40.0	—
	40.0-44.9	6	—	9	—	S. D. 3.5		S. D. 3.2	
	45.0-49.9	5	—	2	—				
	Basophiles								
30-day	9.0-10.9	1	—	3	—				
	11.0-12.9	—	—	10	—				
	13.0-14.9	6	—	11	—	Mean 14.9	—	Mean 12.9	—
	15.0-16.9	4	—	2	—	S. D. 2.01		S. D. 2.2	
	17.0-18.9	2	—	—	—				
	19.0-20.9	—	—	—	—				
	Chromophobes								
	35.0-39.9	6	—	2	—				
	40.0-44.9	5	—	4	—	Mean 40.9	—	Mean 47.1	—
	45.0-49.9	2	—	14	—	S. D. 3.5		S. D. 4.2	
	50.0-54.9	—	—	6	—				

The quantitative data are arranged in statistical form. The frequency distribution, the mean and the standard deviation of the various groups are indicated.

trated. The mature females were sacrificed at 15 and 30 day intervals after operation. Forty-one of these rats received from 25 to 75 units of an extract of pregnancy urine† daily throughout the 15 day castration period or for the last 15 days of the 30 day castration period in those rats which were sacrificed after 30 days. All immature rats were between 25 and 30 days old at operation, and were sacrificed 15 days after operation. Sixteen received 25 units of pregnancy urine extract daily for the entire period; 15 littermate sisters served as controls.

At autopsy the pituitaries were weighed and fixed in Regaud's fluid. Serial sections of all glands were cut. Complete cell counts were made on 5 sections from each of the 90 glands. A total of 331,056 cells were counted.

The quantitative results of these studies are presented statistically in Table 1. (Frequency distribution, means and standard deviations are given). Analysis of this table reveals that the percentages of the various cell types in the anterior pituitaries of the injected rats were almost identical to the percentages of these cells in the anterior pituitaries of the control rats castrated for a similar period of time. Morphologically the anterior pituitaries of the injected rats and those of the controls appeared identical. From the results of these experiments we feel justified in concluding that extracts of pregnancy urine are without action on the anterior pituitaries of castrated female rats.

7608 C

Comparative Quantitative Effects of Castration in Mature and Immature Female Rats.*

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Ellison and Wolfe[1] have reported that in the anterior pituitaries of castrated mature female rats there is an increase in the percentages

† Follutein was furnished by E. R. Squibb and Sons through the courtesy of Dr. J. J. Durrett.

* These studies have been supported by the Committee for Research in Problems of Sex of the National Research Council, the Committee for Scientific Research of the American Medical Association, and the Division of Medical Sciences of the Rockefeller Foundation.

[1] Ellison, E. T., and Wolfe, J. M., *Endocrinology*, 1934, **18**, 555.

TABLE I.

Percentage of Cells	Frequency Distribution			Mean-Standard Deviation (Percent)		
	15-day castrates		30-day castrates	15-day castrates		30-day castrates
	Mature	Immature	Mature	Mature	Immature	Mature
Eosinophiles						
30.0-34.9	6	4	—	Mean 37.1	Mean 37.1	Mean 41.2
40.0-44.9	4	6	15	S. D. 3.1	S. D. 3.1	S. D. 3.8
45.0-49.9	—	—	7			
Basophiles						
5.0- 6.9	3	—	—			
7.0- 8.9	9	—	—			
9.0-10.9	11	—	4			
11.0-12.9	2	1	10	Mean 9.0	Mean 15.8	Mean 13.6
13.0-14.9	—	8	17	S. D. 1.6	S. D. 1.9	S. D. 2.0
15.0-16.9	—	13	6			
17.0-18.9	—	8	2			
19.0-20.9	—	—	—			
21.0-22.9	—	1	—			
Chromophobes						
35.0-39.9	—	—	8			
40.0-44.9	—	9	9			
45.0-49.9	2	20	16	Mean 54.3	Mean 46.3	Mean 45.0
50.0-54.9	13	2	6	S. D. 3.4	S. D. 2.6	S. D. 4.9
55.0-59.9	9	—	—			
60.0-64.9	1	—	—			
	—	—	—			
No. of rats	25	31	39			

The quantitative data are arranged in statistical form. The frequency distribution, the mean and the standard deviation of the various groups are indicated.

of the basophiles to a mean level of 9% in the 15-day castrates and 13.6% in the 30-day castrates. A few signet-ring castration cells were also found in the anterior pituitaries of the 30-day castrates. In further studies[2] we have pointed out that injection of pregnancy urine extracts† are without action on the pituitaries of mature female rats castrated for 15 and 30 days. By adding the 2 series of injected and uninjected animals together, we have to date studied and made cell counts on the anterior pituitaries of 25 mature females castrated for 15 days and 39 castrated for 30 days. These data are arranged in statistical form in Table I, in which the frequency distribution of the percentages of the various cell types, the mean and the standard deviation are given. In addition, 31 immature female rats were castrated when between 25 and 30 days of age and sacrificed 15 days later. Sixteen of these animals received injections of pregnancy urine extracts. Since these extracts were without action on the anterior pituitary,[3] data from these 31 rats are considered together and arranged statistically in Table I. For non-castrated controls we have to date made cell counts on the anterior pituitaries of 69 mature female rats killed at various periods of the oestral cycle, and 31 immature female rats killed between 25 and 35 days of age. The mean levels of the eosinophiles in the anterior pituitaries of the mature and immature controls were 33.6 and 36.1%, respectively. The standard deviations (S. D.) were 4.5 and 3.9, respectively. The mean level of the basophiles in the mature controls was 4.8% (S. D. of 1.2) and in the immature controls was 7.2% (S. D. of 1.3). The mean levels of the chromophobes were 61.9% (S. D. of 4.2) in the mature controls and 55% (S. D. of 3.2) in the immature controls.

Analysis of Table I reveals that in 25 mature female rats castrated for 15 days, the mean level of the basophiles was increased to 9% and in 39 mature females castrated for 30 days, the mean level of the cells was increased to 13.6%. (The mean level in 69 non-castrated mature females was 4.8%). Further analysis reveals that in 31 immature rats, castrated before they were 30 days of age and sacrificed 15 days after removal of the ovaries, the mean level of the basophiles was increased to 15.8%, a mean percentage which was slightly higher than that found in mature female rats

[2] Wolfe, J. M., Ellison, E. T., and Rosenfeld, Louis, *Anat. Rec.*, 1934 supplement, 58-94.

† Follutein was furnished by E. R. Squibb and Sons through the courtesy of Dr. J. J. Durrett.

[3] Wolfe, J. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 184.

castrated for a period of time which was twice as great (30 days). Study of the frequency distribution of these cells in the mature and immature groups will show the individual variations in the different groups and demonstrate in a clear-cut fashion that the factor of age must be considered seriously when one is studying the reaction of the anterior pituitary of the female rat to castration. It is impossible at the present time to give an explanation of this interesting finding. It is pointed out that the initial levels of the basophiles are much higher in normal immature female rats than they are in mature females killed during the various phases of the oestral cycle. Also in the anterior pituitaries of immature female rats a great majority of the basophiles are completely filled with granules, while in the mature female these cells undergo cyclic changes in their granular content.

Reference to Table I will show that the mean level of eosinophiles in 39 female rats castrated for 30 days was 41.2% while the mean level of these cells in 69 mature normal female rats was 33.6%. This would indicate that in this group of castrates there was some increase in the eosinophiles. However, it is important to point out that occasionally the level of these cells in non-castrated females was slightly higher than 40%. Since the mean level of these cells in the 30-days castrates was only slightly above the upper limits of normal for normal females it seems questionable to conclude without additional data that castration in mature female rats results in an increase in the eosinophiles.

7609 C

Suspension Stability of Erythrocytes in Solutions of Gum Acacia.*

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It is stated that the suspension stability of erythrocytes is dependent upon variations in the albumin, globulin, and fibrinogen content of plasma.[1, 2] An increase in the fibrinogen, or globulin, tends to diminish the stability.[3] A careful study of this phenome-

* Assisted by a grant from the Christine Breon Fund.

1 Fahraeus, R., *Acta Med. Scand.*, 1921, **40**, Suppl.

2 Westergren, A., Juhlin-Dannfelt, C., and Schnell, R., *Acta Med. Scand.*, 1932, **77**, 469.

non[4] reveals that although there appears to be an association between an increase in blood serum globulins and a diminished suspension stability, the real causative factor is an agglutinant which may or may not be associated with the globulins. The sedimentation of red blood corpuscles in the presence of this agglutinant, found in physiologically altered blood, may be paralleled experimentally in solutions of gum acacia.

When solutions of gum acacia are administered intravenously to human subjects it is noted that the blood becomes difficult to smear, and that the suspension stability of the erythrocytes is diminished. Similar phenomena may be observed when blood is mixed with gum acacia solutions *in vitro*.

Method. Human subjects with normal and shortened sedimentation rates, and subjects receiving gum acacia solutions intravenously were chosen for this study. All sedimentation experiments were done using the Friedlander tube and recording the time necessary for the column of erythrocytes to settle 18 mm. A 20 cc. sample of venous blood was withdrawn and oxalated. A sedimentation test was done directly on this sample, and another was done using the Lenzenmeier[5] technic. The remainder of the sample was separated by centrifugation and the corpuscular moiety washed with 3 changes of Locke's solution. Then 0.2 cc. of washed corpuscles were resuspended in 0.8 cc. of the following menstrua: plasma,

TABLE I.
Sedimentation Rate Expressed in Minutes for a Column of Cells to Settle 18 mm.

		Slowly Sedimenting Blood 4 samples	Rapidly Sedimenting Blood 9 samples
S.R. Lenzenmeier technic		192	24
S.R. oxalated undiluted specimen		186	14
S.R. cells resuspended in plasma	E.V.	44	7
S.R. " " " Locke	E.V.	719	726
S.R. " " " 1% acacia	E.V.	66	55
S.R. " " " 2% "	E.V.	11	11
S.R. " " " 3% "	E.V.	4.7	3.5
S.R. " " " 4% "	E.V.	5	3.8
S.R. " " " 6% "	E.V.	6.5	4.6
S.R. " " " 8% "	E.V.	9.2	7
S.R. " " " 10% "	E.V.	36	30

S.R. = Sedimentation rate.
E.V. = 20% cell volume.

[3] Westergren, C., Theorell, H., and Widstrom, G., *Z. f. d. g. Exp. Med.*, 1931, **75**, 668.

[4] Lucia, S. P., Gospe, S., and Brown, J. W., to be published.

[5] Lenzenmeier, G., *Arch. f. Gynaekologie*, 1920, **113**, 608.

Locke's solution, 1, 2, 3, 4, 6, 8 and 10% acacia in buffered Locke's solution, the resulting cell volumes being 20% or 2.15±0.1 million corpuscles per c.mm. The experiments are therefore corrected for a standard volume.[6] In all resuspension experiments the syringes were rinsed in 10% potassium oxalate solution.

Results. Table I records the results, expressed as averages, obtained on 13 different samples of blood.

It will be noted that although the sedimentation rates of the 2 series differ considerably, when suspended in their respective plasmas, there are no marked differences when these same cells are resuspended in Locke and acacia solutions under standard conditions of volume.

The following experiment was done in order to determine, macroscopically, the time of rouleaux formation, and the rapidity of sedimentation. For this purpose a stop-watch was used.

TABLE II.

The Sedimentation Rate Expressed in Minutes for a Selected Sample.

S.R. Lenzenmeier technic 235′
S.R. oxalated undiluted specimen 176′
S.R. cells resuspended in plasma E.V. 50′ RBC's = 2,220,000/cmm.
S.R. ″ ″ ″ Locke E.V. 450′ RBC's = 2,110,000/cmm.

S.R. acacia in Locke	Fall in mm.	1%	2%	3%	4%	6%	8%	10%
Rouleaux formation begins at		2′ 0″	1′10″	1′15″	1′02″	1′03″	2′25″	2′ 0″
	mm.							
	1	5′50″	1′25″		1′15″	1′58″	3′45″	
	2	9′35″	2′19″	1′30″	2′14″	2′44″	4′15″	
	3	12′20″	3′08″			2′55″	4′30″	4′50″
	4	13′50″	4′25″			3′10″	4′45″	5′15″
	5	14′45″	4′39″	2′30″	2′39″	3′17″	4′57″	5′40″
	6	17′45″	4′52″	2′45″		3′24″	5′10″	
	7	20′30″	5′29″			3′31″	5′19″	6′15″
	8	23′15″	5′58″			3′46″	5′26″	
	9	26′25″	6′27″				5′33″	
	10		6′42″		3′04″		5′40″	
	11		6′50″				5′47″	6′45″
	12	34′10″	7′20″	3′30″	3′19″	4′26″	5′54″	6′55″
	13		7′50″				6′02″	
	14		8′23″					
	15		8′43″					7′25″
	16		9′05″					
	17		9′25″					
	18	56′0″	10′00″	4′15″	3′54″	4′56″	6′52″	8′10″

An examination of Table II reveals that at least one minute elapses before rouleaux formation is initiated. Sedimentation begins slowly at first and then progresses with an accelerated velocity followed by retardation. In rapidly sedimenting bloods the rate of sedimentation is not a rectilinear function.

[6] Walton, A. C. R., *J. Lab. and Clin. Med.*, 1933, **18**, 711.

When samples of corpuscles in acacia solutions are examined microscopically, it is noted that rouleaux formation is slow and the clumps small in the 1% solution; rapid with large clumps in the 10% solution.

Summary. The factors responsible for alterations in the suspension stability of erythrocytes is independent of the corpuscles. Solutions of gum acacia reduce the suspension stability of the blood. A solution of 3 or 4% of acacia is the minimal amount that will produce the maximal instability of erythrocyte sedimentation. The rapidity of formation and the size of agglutinated erythrocyte masses progresses parallel to the increase in concentration of acacia in solution. The curve of sedimentation for rapidly sedimenting bloods is not a rectilinear function.

7610

No Anti-Hormones Against Estrin.*

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In 1934, Collip indicated that there may exist anti-hormones, or substances produced as a result of continued hormone injection which tend to counteract the effect of the hormone. In the preparations which he used, however, the possibility of protein being present could not, with absolute certainty, be excluded. The so-called anti-hormone might then possibly have been an antibody, although evidence opposing this belief was presented.

It seemed to us desirable to investigate the possibility of anti-hormone production against estrin since we would here be dealing with a substance certainly not a protein, and also because we found in earlier work[1] that continued estrin administration did not cause a continued increase in size of the uterus, but rather the reverse, which might conceivably be explained on the basis of anti-hormone production.

Two series of experiments were performed. In the first, 3 groups of 10 rats each were used, one group normal, mature females, the

* This investigation was supported in part by a grant from the National Research Council, Committee on Problems of Sex.

[1] Spencer, Jack, D'Amour, Fred E., Gustavson, R. G., *Am. J. Anat.*, 1932, **50**, 129.

second, normal, mature males, and the third, ovariectomized females castrated some 9 months previously. All animals were injected once per day with 5 rat units of estrin during the first 3 weeks and with 20 rat units for the next week. In the second series, groups of the same size and kind of animals were used. These were given 5 rat units of estrin during the first 3 weeks and 20 rat units daily for the following 5 weeks. The material was highly purified, non-crystalline estrin from human pregnancy urine, carefully assayed by the Coward-Burn method. After the injections were completed the animals were allowed to rest for 4 days to permit excretion of residual estrin. They were then etherized, the carotid cannulated and the blood withdrawn through the cannula into a syringe. The blood was permitted to clot and between 3 and 4 cc. of serum obtained per rat.

In determining the ability of this serum to neutralize the effect of estrin, the following procedure was adopted. One hundred newly ovariectomized rats were used, one group was injected with estrin only, one group with estrin plus serum in the same amounts and from rats of the same kind as the injected animals and the third with estrin plus the serum from the injected animals. Those given serum received one-third the total amount at the same time they received the estrin, one-third 12 hours later and the remainder 24 hours after the first injection. The serum given represented approximately the total amount obtained from one donor rat and varied between 3 and 4 cc. The estrin was given in oil subcutaneously and the serum intraperitoneally. All rats were given 2 Coward-Burn rat units of estrin. (One Coward-Burn rat unit repre-

TABLE I.

No. of Rats Injected	Material Injected	% in Estrus
50	2 R.U. Estrin only	88
10	2 " " plus serum from non-injected males	80
10	2 " " " " " males injected for 1 mo.	100
10	2 " " " " " " " " 2 mo.	90
10	2 " " " " " non-ovariectomized, non-injected females	90
10	2 " " " " " non-ovariectomized females injected for 1 mo.	80
10	2 " " " " " non-ovariectomized females injected for 2 mo.	80
10	2 " " " " " ovariectomized, non-injected females	80
10	2 " " " " " ovariectomized females injected for 1 mo.	90
10	2 " " " " " ovariectomized females injected for 2 mo.	80

sents the amount of estrin necessary to bring 50 out of 100 rats into estrus.)

Table I shows the results obtained.

Conclusions. We conclude that the results obtained according to the method used do not indicate the formation of any anti-hormone against estrin.

7611 P

Effect of Various Dietary Principles on Lactation in Rats.

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It has been known for some time that milk production can be materially increased by dietary treatment. This increase was first accomplished by increasing the protein content of the diet, then later by using good quality animal proteins such as liver. The vitamins, especially the B complex, are of considerable importance as well.

In the present study the growth of approximately 350 litters of suckling rats was used as a criterion of milk production. Only the growth period from the fourth to the seventeenth day of life was considered. The logarithmic functions of the daily weight of litters of 6 were plotted against time. The slopes of the resultant curves were compared, and expressed as lactation indices.

The basal diet of the lactating rats consisted of casein, 15 parts; salt mixture, 5; agar agar, 2; starch, 76; lard, 14; wheat germ oil, 5 drops per day; cod liver oil, 6 drops per day; and irradiated yeast, 3 gm. per day. To this basal ration were added daily supplements of various fractions of liver or egg. When dried substances such as amino acids were used they were incorporated in the basal ration replacing part of the casein.

The lactation promoting factor was found to be present in considerable amounts in the following: liver, egg, water extract of autolysed liver or egg; 25% level of casein, Witte's peptone, blood fibrin, cystine. The best results were obtained when one part of cystine replaced one part of casein in the basal ration. This is a rather low protein diet for a rat, especially a lactating rat, and the results suggest that cystine plays a specific rôle in stimulating milk production either as such or as a constituent of glutathione which in turn may influence the production of milk.

Poor results were obtained with the following: extra lard, liver or egg fat; the unsaponifiable fractions of liver or egg fat, lecithin, and extra wheat germ oil.

7612 C

Spontaneous Activity, Direct and Indirect Measures of Sexual Drive in Adult Male Rats.

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Numerous experiments[1–5] have demonstrated a significant relationship between the level of spontaneous activity in male rats, as measured by revolving drums, and the presence or the absence of the testes. Castration is followed by a marked decrease in spontaneous activity. Although the reduction is somewhat less striking when castration is performed on infantile males than when it is performed on fully developed males,[6] there is at either age a decline in spontaneous activity that may be ascribed to the loss of gonadal secretions.

A clear relationship has also been shown to exist between copulatory behavior and the presence or absence of the testes. Young male rats castrated prior to puberty seldom if ever copulate or display aggressive sexual behavior toward receptive females; furthermore, adult males, although copulating for some weeks or even months after castration,[7] soon show a measurable reduction in sexual drive as measured either by direct tests of copulatory frequency or by obstruction tests.[8] These facts indicate that reduction in spontaneous activity and reduction in quantitative expressions of sexual vigor go hand in hand in castrated males when the latter are compared with normal males. They also suggest the possibility of using the revolving drum technique to study sexual drive in normal

1 Hoskins, R. G., *Am. J. Physiol.*, 1925, **72**, 324.

2 Wang, G. H., Richter, C. P., and Guttmacher, A. F., *Am. J. Physiol.*, 1925, **73**, 581.

3 Richter, C. P., and Wislocki, G., *Am. J. Physiol.*, 1928, **86**, 651.

4 Slonaker, J. R., *Am. J. Physiol.*, 1930, **93**, 307.

5 Richter, C. P., *Quart. Rev. Biol.*, 1927, **2**, 307.

6 Richter, C. P., *Endocrinology*, 1933, **17**, 445.

7 Stone, C. P., *J. Comp. Psychol.*, 1927, **7**, 369.

8 Nissen, H. W., *Genet. Psychol. Monog.*, 1929, **5**, 451.

males; however, it should be borne in mind that an instrument that adequately registers wide variations in a given phenomenon may be wholly inadequate when fine discriminations are required. The usefulness of revolving drums in studying sexual drive in intact animals must be determined by special experiments.

In the present study, we have determined the relationships between spontaneous activity of 24 male, albino rats confined in revolving drums and their numerical scores on 2 reliable tests of sexual drive; namely, (1) frequency of their copulations in direct tests with highly receptive females, and (2) frequency of their attempts to overcome an obstruction separating them from a receptive female.[9] The males were between 6 and 7 months of age. Their average weight was 280 gm. and the range of weights was from 190 to 320 gm. They had been reared on the Steenbock diet, supplemented about twice weekly by lettuce. All were in a very fine state of nutrition and entirely free from skin parasites. When the males were approximately 5 months old they were released in a laboratory room with floor space of approximately 16 square meters. With them were placed 2 dozen adult females in order that they might have unrestricted opportunity for sexual congress. From time to time, non-pregnant females were substituted for the pregnant ones during the period of cohabitation which lasted approximately one month.

Finally, the males were transferred as a group to revolving drums made available through the courtesy of Prof. J. R. Slonaker of the Physiology Department, Stanford University. The males were kept in the revolving drums for a total of 5 weeks, one week devoted to preliminary adjustment to the new cage situation and 4 weeks devoted to the study of spontaneous activity. At the end of each week the males were shifted to different drums in order to distribute at random the unmeasured influences of possible adventitious factors such as small differences in room temperatures and air currents, the proximity of sluggish or active animals that might act as sedatives or as stimulants to certain individuals, or differences in the inertia of the cages themselves. So far as we have been able to ascertain from published reports by Prof. Slonaker, the amount of the activity of our males appears to be essentially like that of his males of similar ages that were allowed to keep the same cage for long periods of time.[10]

[9] Jenkins, T. N., Warner, L. H., and Warden, C. J., *J. Comp. Psychol.*, 1926, **6**, 361.

[10] Slonaker, J. R., *Am. J. Physiol.*, 1926, **77**, 503.

The tests* for copulatory frequency were made a few days after the males were taken out of the revolving drums. Each male was tested between the hours of 8 and 11 p.m. in his home cage with one highly receptive female. The number of copulations and the number of ejaculations were recorded during each sub-test period. The total test consisted of 4 sub-tests, each of 15 minutes duration. The first and second sub-tests came on the same evening with a 20-minute pause between tests; the third and fourth sub-tests were conducted exactly as the first pair of tests and were administered one week later. During the interim between the first and the second pair of tests the males were kept apart from the females.

Tests* with the Columbia Obstruction Apparatus[9] were begun one week after the last of the direct tests for copulatory frequency. With this technique of measuring sexual drive the number of contacts with the electrified grid and the number of crossings to the incentive compartment are taken as the measures of sexual drive. The tests were conducted on 2 nights one week apart. Each night's test consisted of 2 sub-tests that were separated by a 5-minute pause. The sub-tests were of 10 minutes duration and during this time the male was given complete freedom to cross the grid separating the entrance compartment and the incentive chamber. He was returned to the entrance compartment immediately after his arrival in the incentive chamber without giving him an opportunity to copulate with the female incentive. Prior to the test proper, the males were given ample opportunity to familiarize themselves with all parts of the obstruction apparatus, to learn how to manipulate the one-way door leading to the incentive chamber, and to learn that a receptive female with which they had been given opportunity to copulate prior to the tests was in the incentive chamber.

As a measure of the spontaneous activity of each male we have used the total number of revolutions of the drum made during the 28-day period in which his records were taken. The copulatory vigor is denoted by the total number of intromissions during one hour of testing and by a composite score that was obtained by adding to the number of intromissions 10 points for each ejaculation. The obstruction test records consist of the total number of times the grid was crossed in 40 minutes, and of a combined score consisting of the number of crossings plus the number of contacts with the grid. In Table 1 will be found the raw data of each of the tests. As can be seen, there is a considerable amount of variation in the scores

* These tests are described in more detail in a forthcoming paper: Stone, C. P., Tomlin, M. I., and Barker, R. G., *J. Comp. Psychol.* (in press).

TABLE I.

Records of Each Male in the Revolving Drum, upon the Test of Copulatory Frequency, and upon the Obstruction Test.

Column 2, total numbers of revolutions for the 28-day period; Column 3, total numbers of intromissions in 4 copulatory sub-tests lasting 1 hour; Column 4, composite score obtained by adding to total number of intromissions 10 points for each ejaculation; Columns 5 and 6, respectively, total numbers of crossings of the electrified grid and sums of crossings and contacts with grid of the obstruction apparatus in 4 sub-tests which lasted 40 minutes.

Animal No.	Drum Revolutions (in 1000's)	No. of Copulations	No. of copulations + 10(No. ejac.)	No. of Crossings	Crossings + Contacts
1	10	74	144	23	61
2	201	82	122	160	192
3	119	62	92	110	166
4	109	80	140	123	173
5	114	89	149	179	193
6	141	34	44	113	174
7	17	77	147	97	138
8	111	62	122	182	202
9	168	46	66	59	118
10	25	0	0	8	9
11	158	60	110	144	203
12	73	53	93	136	203
13	196	64	104	74	94
14	49	103	183	148	156
15	25	18	18	16	20
16	215	66	126	55	145
17	29	45	45	6	20
18	233	52	82	85	133
19	48	53	113	291	300
20	86	50	120	228	252
21	93	30	30	29	38
22	94	57	137	83	100
23	91	50	70	177	187
24	24	55	145	127	152
Mean	101.25	57.50	101.67	110.42	144.17
S.D.	66.26	21.60	45.72	71.44	73.42

from animal to animal, but there is little tendency for the values from the different tests to vary together. This latter point can be verified by inspecting the correlation coefficients of Table II. In

TABLE II.

Product-Moment Correlations.

	Crossings	Contacts + Crossings	Copulations	Copulations + 10(No. ejac.)
Revolutions in drum	.084	.269	.177	.032
Crossings			.402	
Contacts + crossings				.539

this table will be found the product-moment correlations between the total numbers of revolutions made in the drums, and scores upon the other tests; here, also, will be found the correlations between the scores made on the copulation and obstruction tests.

There is no evidence of a significant relation between the number of revolutions in the drums and scores upon either the obstruction apparatus or the direct test. Applying Fisher's test of the significance of correlation coefficients[11] to the correlation between revolutions and contacts plus crossings, the largest coefficient, it appears that there is a probability of 30 in 100 that such a correlation might arise by chance in an uncorrelated population. Obviously no significance can be attached to a coefficient with such a low reliability.

In contrast to this finding, there is clear evidence of some community of function between the direct copulatory and the obstruction test scores.

The reliability of the scores denoting spontaneous activity has been determined for the present data by correlating the sums of revolutions on the odd with the sums of revolutions on the even days of the total test. This correlation is .80 and becomes .88 when the Spearman-Brown formula is used to obtain an estimate of the reliability of the total test.[12] Previously we have found that the reliabilities of the direct copulatory tests and the obstruction test are, respectively, .91 and .89. In view of the reliability of each of these tests, we may assume that the failure to obtain significant correlations between the revolving drum scores and the other tests cannot be ascribed to errors of measurement of the tests.

In conclusion, then, we find no reliable evidence that there is a true relation between the revolving drum activity of normal adult male rats and the best estimates of sexual aggressiveness now available. As experiments with castrates suggest, however, a relatively low correlation might be found if exceedingly large differences in sexual drive existed among the animals being studied.

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11 Fisher, R. A., *Statistical Methods for Research Workers*, 3d ed., Edinburgh: Oliver and Boyd, 1930, xiii+283.

12 Kelley, T. L., *Statistical Method*, New York: Macmillan, 1923, xi+390.

7613 C

Fasting Blood Carotene Level in Normal and Diabetic Individuals.

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In the course of a study on the effect of the oral administration of carotene on the blood carotene of normal and diabetic individuals, we investigated the fasting blood carotene level in these 2 groups. The number of figures available on this subject are still somewhat limited, and are mostly single observations taken at random.[1, 2, 3] Rabinowitch[4] in a study of carotinemia in diabetes does not report the figures for blood carotene as mg. % but in units, so that the actual figures are difficult to compare with those of other observers.

The diabetic patients studied were on a known diet of carbohydrate, protein and fat. The normals were on a mixed diet from which carrots were omitted, as was the case in the diabetic diets. Fasting bloods were taken. The serum carotene was estimated by the method of White and Gordon[2] and is reported in mg. %. Blood cholesterols were done by the Bloor colorimetric method.[5] Nine normals were observed (Table I) and 19 diabetics (Table II). At least 3 determinations were done on each patient. In all, 35 observations were made on normals and 83 on diabetics. The results on the normals analyzed according to Dunn[6] show the average fasting normal

TABLE I.
Average Blood Carotene and Cholesterol in Normals.

		Average		Upper Limit		Lower Limit	
Age	Sex	Carotene mg. %	Chol. mg. %	Carotene mg. %	Chol. mg. %	Carotene mg. %	Chol. mg. %
50	M	.122	158.	.122	172.	.122	148.
45	M	.077	176.	.081	192.	.068	164.
48	M	.122	190.	.122	217.	.122	176.
44	M	.108	163.	.108	227.	.108	145.
22	M	.136	172.	.122	179.	.095	167.
34	M	.054	165.	.054	180.	.054	152.
22	M	.113	176.	.135	208.	.108	156.
26	M	.148	172.	.162	192.	.122	161.
22	M	.153	161.	.176	217.	.122	122.

1 Connor, C. L., *J. Biol. Chem.*, 1928, **77**, 619.

2 White, F. D., and Gordon, E. M., *J. Lab. and Clin. Med.*, 1931-32, **17**, 53.

3 Stoner, W. C., *Am. J. Med. Sc.*, 1928, **175**, 32.

4 Rabinowitch, I. M., *Arch. Int. Med.*, 1930, **45**, 586.

5 Bloor, W. R., Pelkan, J. F., and Allen, D. M., *J. Biol. Chem.*, 1922, **52**, 191.

TABLE II.
Average Blood Carotene and Cholesterols in Diabetics

		Aver.		Upper Limit		Lower Limit		Diet			
Age	Sex	Carotene mg. %	Chol. mg. %	Carotene mg. %	Chol. mg. %	Carotene mg. %	Chol. mg. %	Carb. gm.	Pro. gm.	Fat gm.	Daily Insulin Units
47	M	.318	272.	.338	308.	.284	232.	250	65	85	30
45	M	.216	204.	.230	223.	.203	194.	200	75	85	30
48	M	.337	211.	.351	229.	.311	194.	250	75	85	75
40	M	.207	241.	.216	250.	.203	227.	180	65	85	15
65	M	.337	251.	.378	294.	.257	222.	180	75	85	none
49	M	.277	178.	.324	225.	.230	156.	220	65	85	8
30	F	.212	214.	.230	227.	.203	200.	200	70	83	50
34	F	.131	189.	.149	217.	.108	161.	280	73	83	15
18	M	.220	218.	.230	263.	.216	176.	200	65	85	60
64	F	.135	250.	.162	296.	.108	200.	220	63	83	8
62	F	.090	217.	.094	253.	.081	194.	150	63	70	none
61	F	.205	302.	.256	378.	.136	264.	200	63	85	"
30	M	.284	235.	.337	312.	.216	200.	160	60	90	28
63	M	.346	196.	.378	221.	.294	181.	165	62	108	none
66	F	.302	323.	.351	347.	.243	301.	150	52	62	"
60	M	.176	166.	.203	222.	.148	143.	200	60	87	22
53	M	.144	222.	.162	248.	.108	208.	142	60	90	none
49	F	.482	269.	.750	329.	.335	219.	174	63	83	12
22	M	.284	290.	.297	306.	.270	258.	245	65	85	40

serum carotene to be 0.109 mg. % with a standard deviation of ±0.104. The limits then would be 0.213 mg. % and 0.005 mg. %. All of our observations fell within these limits so that the figures may be considered significant. In the diabetics the average fasting serum carotene was 0.262 mg. % with a standard deviation of ± .112. The limits are then 0.374 and 0.150 mg. %. Seventy-four percent of the figures fall within the average ± the standard deviation. Ninety-five percent fall within twice and 99% within 3 times the standard deviation.

It is clear from these observations that the fasting blood carotene is higher in diabetics than in normals. The average of the diabetics being 0.262 mg. % as compared to 0.109 mg. % for the normals. The average cholesterol in the normals was 178 mg. % as compared to 233 mg. % in the diabetics.

[6] Dunn, H. L., *Physiol. Rev.*, 1929, **19**, 275.

7614 C

Swelling of the Muscles of Adrenalectomised Rats.*

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Following a suggestion of Viale and Bruno,[1] that the changes in water content of the tissues of adrenalectomised animals may be due to an increase in permeability, Winter and Hartman[2] carried out experiments on the swelling and shrinking of muscles from normal and adrenalectomised rats in balanced salt solutions of various strengths. They conclude that water enters or leaves muscles from adrenalectomised animals more readily than it leaves or enters normal muscles, and regard this as evidence of an increased permeability following the adrenalectomy. Since a hormonal control of tissue permeability, if demonstrated, would be of extreme importance in general physiology, we have attempted to reproduce Winter and Hartman's results.

The method used was essentially that of Winter and Hartman, except that we confined ourselves to determining the course of the swelling curves of muscles in a hypotonic solution, instead of investigating shrinking curves in hypertonic solutions as well. The latter are notoriously irregular, and are not suitable for analysis. The rats used were adrenalectomised at 30 days of age, and in all but a few cases were killed in the terminal stages of adrenal insufficiency, as indicated by a fall in weight and body temperature, asthenia and oftentimes prostration. In a few cases the rats were killed in slightly earlier stages of adrenal insufficiency. The animals survived from 3 to 32 days after the operation, the average time of survival being 9 days. The average weight at the time they were killed was 63 gm. The control rats were from the same stock, and as nearly as possible of the same age.

The animals were killed by a blow on the head, and both gastrocnemii dissected out entire. The muscles were immersed in the Locke's solution described by Winter and Hartman (pH = 7.3), diluted so as to be hypotonic (70 parts solution plus 30 parts of

* We are indebted to Mr. C. E. Tobin for assistance in the preparation of animals.

1 Viale, G., and Bruno, A., *Compt. Rend. Soc. de Biol.*, 1927, **97**, 261.

2 Winter, C. A., and Hartman, F. A., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 207.

water). They were weighed before immersion and after intervals of 3, 6, 10, 15, 25, 40, and 60 minutes on a torsion balance, which allows very rapid weighing. There is little point in continuing observations beyond about 60 minutes, for the swelling curves become very irregular.

The average results for 40 muscles from 20 adrenalectomised animals and for 40 muscles from 20 controls are shown in Table I.

TABLE I.

	% gain in weight						
	3′	6′	10′	15′	25′	40′	60′
Normals	106.1	109.1	111.8	114.7	118.3	122.8	127.1
Adrenalectomised	104.1	106.9	109.1	112.1	114.9	118.4	122.3
Difference	2.0	2.2	2.7	2.6	3.4	4.4	4.8
S.E. of difference	0.57	0.68	0.87	1.10	1.28	1.37	1.43

The table shows that the muscles of the adrenalectomised rats take in water at a slower rate than do those of the control animals, a conclusion which is exactly the opposite from that of Winter and Hartman. Winter and Hartman do not give figures to show that the differences in swelling rate which they observed (in the opposite direction) were statistically significant, but there is no doubt about the differences shown above. If differences in the form of the swelling curves obtained in experiments such as these are to be taken as indicating differences in permeability, our conclusion would have to be that the muscles of adrenalectomised animals are less permeable to water than those of normal animals: there are, however, several reasons for attributing the differences to other than permeability changes.

1. Throughout the experiments we observed that heavier muscles tended to swell less rapidly than lighter ones, even when the swelling was expressed in each case as a percentage of the initial weight. This is to be expected on the grounds that the heavier muscles have a smaller surface/volume ratio. It so happened that our control muscles were somewhat lighter on the average than the muscles from the adrenalectomised animals, and this difference in weight is probably partly responsible for the slower swelling of the latter.

2. When muscles of equal weight from normal and adrenalectomised animals were compared, the rate of swelling of the control muscles was again found to be greater than that for the muscles from the adrenalectomised rats, although the differences were smaller than before. Taken individually, indeed, (as in the table

above) only one such difference exceeded twice its standard error, but all the differences were in the same direction, and the odds against this occurring by pure chance are enormous. A simple explanation exists, however, for this greater rate of swelling in the case of the controls, for the muscles from the control animals contained a smaller percentage of water than those of the operated animals.[3-5] If we suppose that the initial tonicity of the control muscles was higher than that of the muscles of the adrenalectomised animals, the former would be expected to swell more rapidly than the latter in any given hypotonic solution. The average water content of our control muscles was 76.6%, and that of our muscles from the operated animals 78.3%; this difference is about of the right order, if reflected in a tonicity change, to account for the different swelling rates observed.

3. While these are the average results for all the muscles used, the swelling curves of individual muscles vary so much that we are very doubtful if this type of experiment can be relied upon to supply information regarding permeability of tissues. For instance, the muscles of the right and left legs of the same animal do not always show the same swelling curve, even when the 2 muscles are of the same initial weight, and we have met with as great differences between the behavior of the paired muscles as between the behavior of normal muscles and those from the adrenalectomised animals.

Finally, it ought to be pointed out that an increase in the permeability of the tissues to water, and this alone, could not account for the increased water content of the tissues after adrenalectomy. The water content is determined, not by the rate of entry of water, but by those conditions which control the final equilibrium, and these might well change without any alteration in permeability being involved.

Conclusions. The conclusion of Winter and Hartman, that adrenalectomy in the rat is followed by an increase in the rate at which water enters the muscles, *i. e.*, by an increase in permeability, is not confirmed. The observation that the muscles of the operated animals contain a greater percentage of water is confirmed. It is pointed out that an increase in permeability to water, even if it were to exist, would not account for the increased water content.

[3] Hartman, F. A., Brownell, K. A., and Lockwood, J. E., *Endocrinology*, 1932, **16**, 521.

[4] Silvette, H., and Britton, S. W., *Am. J. Physiol.*, 1933, **104**, 399.

[5] Hartman, F. A., *Annals Int. Med.*, 1933, **7**, 6.

7615 C

Effect of Feeding Thyroid on Anterior Hypophysis of the Female Albino Rat.*

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It has been reported that feeding thyroid to rats increases the gonad-stimulating complex of the anterior hypophysis.[1, 2] In an attempt to obtain a morphologic basis for this increased physiologic effect, we have studied serial sections of the anterior pituitaries of 28 female rats which were fed varying amounts (250 to 1,000 mg.) of thyroid daily for periods of 30 to 71 days. Twelve of these rats were placed on experiment when immature; the others were fully mature when thyroid feeding was initiated. Confirming studies of other investigators,[2, 3, 4] it was found that feeding thyroid in sufficient amounts suppressed the oestral cycle resulting in prolonged periods of dioestrus. However, it was also found that by varying the dosage of thyroid the oestral cycle could be regulated almost at will. In rats receiving the smaller amounts, the oestral cycles were usually regular, occasionally interspersed with cycles the length of pseudo-pregnant cycles. Weichert and Boyd[5] consider that such cycles represent a true lutein phase. Larger dosage resulted in prolonged periods of dioestrus, ranging up to 40 days. In some animals, oestrus was suppressed for periods of time ranging up to 72 days, which covered the entire period of observation. The heavier thyroid dosages fed in these experiments were sufficient to decrease the growth rate slightly, but never to a marked degree. Animals receiving smaller dosages exhibited a normal rate of growth.

At autopsy it was found that the weight of the ovaries and the pituitaries of those animals which had exhibited oestral cycles of normal or pseudo-pregnant length were normal in appearance and weight. In those rats in which the oestral cycle had been suppressed to a more marked degree, the ovaries were decreased in weight and

* These studies were aided by grants from the Committee for Scientific Research of the American Medical Association and the Division of Medical Sciences of the Rockefeller Foundation.

1 Evans, H. M., and Simpson, M. E., *Anat. Rec.*, 1930, **45**, 215 (Supplement).

2 Van Horn, H. N., *Endocrinol.*, 1933, **17**, 152.

3 Reiss, M., and Pereny, S., *Endokrinologie*, 1928, **2**, 181.

4 Weichert, C. K., *Physiol. Zool.*, 1930, **3**, 461.

5 Weichert, C. K., and Boyd, R. W., *Anat. Rec.*, 1933, **58**, 55.

the accessory reproductive organs approached a castrate condition. In these rats the weight of the pituitary was also decreased. Considering the group as a whole, the mean pituitary weight was 7.8, while that in 69 control females was 10.9 mg.

The pituitaries were prepared for study by means already described.[6] The ovaries and accessory organs were fixed in Bouin's fluid and the sections stained with hematoxylin and eosin. Serial sections of the pituitary and the ovaries were cut. Cell counts were made on representative sections of the anterior pituitary; a total of 89,535 cells was counted.

Histologically the ovaries and the accessory organs of those animals in which the oestral cycles were moderately to markedly suppressed exhibited definite changes. The ovaries exhibited a normal number of follicles, mostly small and moderate in size, but a few were as large as those found in the ovaries of late prooestral rats. However, much follicular atresia was evident which involved most of the follicles. The large amount of interstitial tissue and the small number of corpora lutea were the outstanding characteristics. Some rats exhibited only one or 2 periods of oestrus, or none at all during the 71 days of observation. In the ovaries of these animals corpora lutea were almost entirely absent, only follicles and interstitial tissue being present. The uteri and vaginae of these rats approached a castrate condition.

Histologically the anterior pituitaries presented certain changes which varied from normal to a degree proportional to that in which the oestral cycle and the reproductive tract varied from normal. The basophiles were increased very slightly in percentage, the mean level in the experimental rats was 5.3%, while the mean level in 69 virgin control rats was 4.8%.

The basophiles were consistently different in appearance from those found in normal females. They were larger and well filled with granules, which took a purple-red stain, varying to a brick red. The granules of basophiles in the anterior pituitaries of normal females take a deep blue stain and often are so closely packed that they give the impression of a dense blue cytoplasm. It is important to emphasize that the basophiles in the anterior pituitaries of the thyroid-fed rats were packed full of granules, while in normal rats these cells exhibit varying degrees of granular depletion. The changes in the basophiles described above were remarkably constant and were most marked in rats in which the oestral cycle had been most markedly suppressed.

[6] Cleveland, R., and Wolfe, J. M., *Anat. Rec.*, 1932, **51**, 409.

In 8 rats which exhibited normal oestral cycles of 5 or 6 days in length, or cycles of pseudopregnant length, the level of the eosinophiles ranged from 30 to 40%, which is the normal level (the mean level of these cells in 69 controls was 33.6%, with a standard deviation of 4.5). The remaining 20 experimental rats exhibited varying degrees of suppression of the oestral cycle. In the anterior pituitaries of these rats the level of the eosinophiles ranged from 24.1 to 28%; such a low level is found only occasionally in normal females.

It is known from the work of the investigators mentioned above that the capacity of the anterior hypophysis to increase the size of the ovaries of immature test animals is increased by thyroid feeding. Our morphologic studies would indicate that there were definite structural changes in the anterior pituitaries of such rats; most notable was some increase in size and definite increase in granular content and change in appearance of the basophilic elements. Changes in the eosinophiles were less constant and where such changes did occur, they were in the nature of a decrease in the percentage of these cells. Our data would indicate, therefore, that the increased capacity of the anterior pituitaries of thyroid-fed rats to increase the weight of the ovaries of immature test rats was probably due to the changes in the basophiles noted above.

One interesting point should be noted; the basophiles in these animals are very similar to those found in the anterior pituitaries of rats killed during the last half of pregnancy. As pointed out previously,[7] during the first 6 days of pregnancy the basophiles are markedly reduced in percentage and granular content, but from the 7th day to delivery they increase gradually in percentage and size and become filled with purple-red granules which vary to brick-red in color. The reason for the similarity of the appearance of the basophiles in these 2 groups of rats is unknown.

The mechanism by which thyroid feeding disturbs the oestral cycle has been discussed fully by Van Horn and Weichert and Boyd. The view that the increased metabolic rate in some way keeps the level of oestrin below the threshold for oestrus is upheld by a certain amount of evidence presented by these workers and by Reiss and Pereny.

Summary. Twenty-eight female rats were fed amounts of desiccated thyroid ranging from 250 to 1,000 mg. daily. The oestral cycle was suppressed to varying degrees, dependent on the dosage of thyroid. The pituitaries were subnormal in weight. Histologically the anterior lobes exhibited certain changes: most notable was

[7] Wolfe, J. M., and Cleveland, R., *Anat. Rec.*, 1933, **56**, 33.

a slight increase in the percentage of the basophiles and a definite increase in the size and granular content of these cells. The granules stained a purple-red which varied to a dull brick-red; in normal female rats (virgin and killed during the normal oestral cycle) the basophiles take a deep blue stain. The changes in the basophiles were most marked in those animals in which the suppression of the oestral cycle was most evident.

7616 C

Variations in Contour of the Records Found in Serial Electrocardiograms of the Dog.

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In the course of some studies we had occasion to take serial electrocardiograms on 3 normal dogs twice a week over a period of 4 months. The dogs were trained to lie on their right sides while the electrocardiograms were taken. The limbs were shaved and flannel bandages soaked in concentrated saline bound around them. Copper wire spiral was then applied tightly over the bandage and connected with the electrodes. The skin resistance was found to be low with this procedure (below 1000 ohms) and no polarization was encountered.

The serial records obtained in each dog over the period of 16 weeks revealed irregular fluctuations in the form of the electrocardiograms. These variations were not progressive, could not be related to environmental factors, and varied within the wide limits illustrated in Fig. 1. These results were obtained in spite of the fact that every effort was made to take the successive electrocardiograms under identical conditions as regards the position and posture of the animal. These normal variations in the electrocardiogram of the dog are probably due to variations in the position of the heart at different times. The relative mobility of the dog's heart as compared to the human is such that it is almost impossible to manoeuver it into exactly the same position time after time. These effects of position involve changes in the amplitude and even direction of all the complexes of the electrocardiogram, especially the T wave. Sim-

* Aided by the Frederick K. Babson Fund for Diseases of the Heart and Circulation and the Max Pam Fund for Metabolic Research.

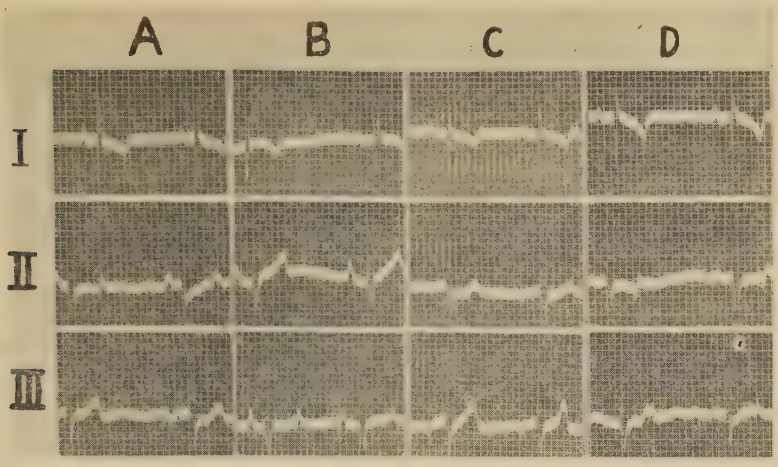

FIG. 1.

Electrocardiograms showing the range of variation observed in a normal dog, under identical conditions, during a period of 16 weeks. These records (segments A-D) are typical of a large number.

ilar variations in the electrocardiogram of one of these animals were observed when repeated records were made with the animal in the normal standing position.

Conclusion. Repeated electrocardiograms in the normal unanesthetized dog, involving repeated preparation of the animal for this procedure, show significant variations. These variations are probably accounted for by the relative mobility of the dog's heart as compared to the human, and must be taken into account in the interpretation of the results of experiments requiring repeated records over a period of days.

7617 P

Behaviour of Isotonic and Hypertonic Solutions in Blood Stream of Normal and Dehydrated Animals.

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The mode of regulation of the blood volume remains still an unsolved problem in spite of the efforts of many investigators.[1-4] It has been pointed out recently that the blood stream does act to some extent as a reservoir in the normal animal.[5] This study was undertaken to evaluate the rôle played by this reservoir action in the regulation of the blood at a constant volume and the effects of dehydration and protein loss. The investigation was divided into 2 parts, the first dealing with effects produced by isotonic solutions introduced in large amounts into the blood stream of normal and dehydrated animals. The second part concerns the results produced by hypertonic solutions.

The effects of isotonic solutions.

Material used—10 normal healthy dogs. Five dogs were used as controls and were allowed free access to water and food. Five were dehydrated by deprivation of water for a standard period of 10 days but food was permitted freely.

Method. 970-980 cc. of 0.9% sodium chloride solution or of 5% glucose solution were injected into the blood stream at a constant rate of 330 cc. per 5 min. Five cc. of blood were withdrawn from the femoral vein before and immediately after injection and at intervals of 15, 60, 180, and 300 minutes. The hemoglobin was determined by the Newcomer method, the plasma volume by the hematocrit method and erythrocyte counts were performed.

Results. The blood vascular system does act as a reservoir, the blood remaining diluted for a period of 5 hours, at which time it has almost returned to its normal state. With isotonic glucose solutions,

* Jessie Horton Koessler Fellow in Experimental Surgery.

1 Bogert, L. J., Underhill, F. P., and Mendel, L. B., *Am. J. Physiol.*, 1916, **41**, 189.

2 Smith, A. H., and Mendel, L. B., *Am. J. Physiol.*, 1920, **53**, 323.

3 Chanutin, A., Smith, A. H., and Mendel, L. B., *Am. J. Physiol.*, 1924, **68**, 444.

4 Erlanger, J., *Physiol. Rev.*, 1921, **1**, 177.

5 Calvin, D. B., Smith, A. H., and Mendel, L. B., *Am. J. Physiol.*, 1933, **105** 135.

the blood dilution shows a progressive diminution to the normal, *i. e.*, there is a uniphasic response. With isotonic sodium chloride solutions, the response is polyphasic, *e. g.*, the maximum dilution may not be reached until one hour after the injection or the initial dilution is followed by concentration and later by a further dilution. The blood of the normal animal retains sodium chloride solutions better than glucose solutions. Dehydration definitely increases the ability of the blood to hold both isotonic sodium chloride and isotonic glucose. Animals, which, by repeated bleedings, have been rendered anemic, as indicated by a definite lowering of the amount of hemoglobin in the blood, are unable to hold water in the blood vascular system even when severely dehydrated. In the normal animal the changes in blood dilution follow closely the changes in urinary secretion. In dehydration, however, following an injection, the blood dilution continues to diminish even although no urine is being excreted, indicating the passage of fluid into the tissues. This study seems to indicate that the major site of electrolyte action is in the extravascular tissues rather than in the blood, and that the blood colloids play a greater rôle than do electrolytes in the maintenance of a constant blood volume: whereas, in the tissues, water storage seems to depend to a greater extent upon electrolyte action.

Effects of hypertonic solutions.

Material—20 dogs. 10 dogs were used as controls and 10 were dehydrated.

Method. Both femoral veins or external jugular veins were exposed under local anesthesia. Morphine hydrochloride was administered before each experiment. The standard injection was 50 cc. of 25% sodium chloride solution. Five cc. of blood were withdrawn from opposite vein before, and at intervals of ½ minute, 3, 7, 10, 15, and 30 minutes following injection. Hemoglobin determination by Newcomer method. Red blood cell count and plasma volume by hematocrit method.

Results. The effect of the injection is instantaneous and results in an increase in the volume of circulating blood. This increase varies from 15.2% to 38.9% according to hemoglobin dilution measurements. The volume increase is of short duration and the norm is reached within 20-30 minutes. In the dehydrated animal the blood volume increase is just as great as in normal animals. Often the response is even greater in the dehydrated state. In dehydration the return of the diluted blood to normal is definitely slower. Repeated hemorrhages leading to a loss of the cellular and protein content of the blood results in a considerable diminution in the response to the standard injection.

7618 P

Microscopic Observations on Circulatory Systems of Living Transilluminated Mammalian Spleens and Parturient Uteri.*

MELVIN H. KNISELY. (Introduced by W. Bloom.)

From Hull Anatomical Laboratory, The University of Chicago.

A. Spleens.—The circulatory system of living spleens of mice, rats, and cats, were studied. The spleens were exposed through a small incision and *vigilantly* protected from minute temperature changes and trauma to prevent general vasomotor upsets in the organ. The spleens were transilluminated, using only the visible spectrum, by means of the previously described[1] quartz rod light. The linings of blood vessels, including sinuses, in living spleens, show as clear refractile borders, not to be confused with peripheral plasma layers of the blood. These linings are as continuous in living spleens as in other organs. Each vessel that I traced connected to both the arterial and the venous system; neither open ends nor blind ends of vessels were found. I saw blood pass through the red pulp via (1) long straight capillaries, (2) via the venous sinus systems and (3) via diapedesis, but I have not yet seen other types of passage. The distribution of blood to various areas and subareas of the red pulp is actively controlled by coordination of the action of powerful sphincter-like segments of branches of the arterial tree. Venous sinuses have a cycle of filling, storage and emptying. During filling the efferent end of the sinus is tightly contracted, whole blood flows into the sinus, plasma filters rapidly out of the sinus into the partitions which are usually termed pulp cords, leaving the sinus distended up to 20 to 50 times its original diameter with solidly packed blood cells. The retention of blood cells lasts from a few minutes to several hours. At emptying, the efferent end of the sinus relaxes suddenly, the packed blood cells emerge in masses, and the sinus decreases in diameter, quickly, until it is but 2 or 3 times the diameter of a red blood cell and then it conducts blood like any other blood vessel. The spleens of digesting animals are large because many sinuses are distended with packed cells. The spleens of exercised or frightened animals are small because most of the sinuses are not storing, but conducting, blood. Administra-

* This research was aided by a grant to The University of Chicago by the Rockefeller Foundation. The assistance and counsel of the members of the Hull Anatomical Laboratory have been invaluable in this work.

[1] Knisely, M. H., *Anat. Rec.*, 1934, **58**, 73.

tion of adrenalin causes the sinuses to empty out their stored blood cells.

During the brief death period of an animal there is a rapid diapedesis of red cells, out of capillaries, in all directions through the red pulp, a disappearance of capillary walls, an intense phagocytosis of red cells, by phagocytes, and rouleaux formation in the sinuses and venules. Agonal changes in the red pulp during the 3 to 5 minutes of the death of the animal may possibly explain the "open" circulatory system as seen in some histologic sections.

B. Parturient uteri.—The living uteri of 6 parturient house mice (*Mus musculus*) were studied, using lens combinations with magnifications up to 100 X. The animals were opened, under light ether, without blood loss, by a para-midline incision, the viscera protected *carefully* from thermal and mechanical trauma, and the uteri and adnexa transilluminated with the quartz rod light. Each of the 2 to 5 branches of the uterine artery supplying a placental site has an especially contractile segment (similar to that in an arteriovenous anastomosis) located near the entrance of the branch into the uterine wall. Contraction and relaxation cycles of these sphincters control the volume of blood supplied to a placental site preceding, during and following parturition. At the site of the lowest attached foetus brief partial contractions of the arterial sphincters alternate with long relaxations. Gradually the duration and degree of contraction change until contractions and relaxations are equal and each contraction completely obliterates its branch's lumen.

The sphincter's contractions later become long and powerful with very brief, partial relaxations. The foetus and foetal side of the placenta at this time are cyanotic while the maternal side of the placenta and uterine wall are blanched and nearly bloodless. The uterine musculature at the level of the attached placenta (Rudolph & Ivy)[2] begins powerful rhythmical contractions. (Smooth muscle of the gut also undergoes strong contractions when its blood supply is cut off.) During a strong, *localized* uterine contraction the foetus and placenta break away and start down the tube, and the sphincters remain tightly closed, preventing hemorrhage. Brief partial relaxations prevent blood loss while a clot is formed at the placental site. The relaxations of the sphincters become longer and the contractions shorter until after half an hour when a clot is well established, the uterus receives a normal blood supply again.

[2] Rudolph and Ivy, *Am. J. Obstet. and Gynec.*, 1930, **19**, 317.

The cycles of adjacent sphincters are out of phase at all times so that the uterine musculature frequently gets a *little* blood through each, even during the detachment of the foetus and placenta. In so small an animal the loss of a little blood is serious. This mechanism conserves blood so well that free blood is hardly ever seen in the uterine lumen. The *localized* interruption of the blood supply of the uterus at a placental site may be one link in the chain of events initiating delivery.

7619 C

Anterior Pituitaries of Infantile Female Rats Receiving Injections of Pregnancy Urine Extract.*

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Many investigators have demonstrated that injections of pregnancy urine or human placental extracts into immature (21-day or above) female rats result in an increase in the size of the ovaries due to follicular maturation and corpus luteum formation. However, subsequent studies of Selye and Collip[1] have revealed that injection of such extracts into infantile female rats (6 to 8 days) fails to cause follicular maturation and development of corpora lutea, but does result in a marked increase in the size of the thecal cells giving rise to thecal corpora lutea.

Collip and associates[2] have found that injection of placental extracts increases the size of the pituitaries of immature female rats (21 days or above) as well as the ovaries. We have confirmed these results, using both extracts of human placentae and pregnancy urine.†[3–5] Histologically, the pituitaries of these rats exhibited a

* These studies were aided by grants from the Committee for Scientific Research of the American Medical Association and from the Division of Medical Sciences of the Rockefeller Foundation.

[1] Selye, H., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 647.

[2] Collip, J. B., Selye, H., Thomson, D. L., and Williamson, J. E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 590.

† Pregnancy urine extract, Follutein, was furnished by E. R. Squibb & Sons through the courtesy of Dr. J. J. Durrett.

[3] Wolfe, J. M., Phelps, D., and Cleveland, R., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1092.

[4] Wolfe, J. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 812.

[5] Wolfe, J. M., *Am. J. Physiol.*, in press.

marked granular loss from the basophiles and a less evident loss of granules from the eosinophiles. Cell counts revealed that the percentages of the basophiles and eosinophiles were decreased, while that of the chromophobes was increased. Since it has been found that injection of pregnancy urine extract brings about a markedly different ovarian effect in infantile rats (6 to 8 days) it seemed of interest to study the anterior pituitaries of such rats.

Litters of female rats 6 days old were used, 2 or 3 animals of each litter serving as experimental animals, the rest as controls. The experimental rats received 25 units of pregnancy urine extract daily. Twenty-four hours after the 10th daily injection one experimental and one control animal were autopsied. The remaining experimental rats received 10 more daily injections and, together with their controls, were sacrificed on the 27th day of life, 24 hours after the 20th injection. Due to the small size of some of the litters, all the controls of this group were not littermates. A total of 64 rats was used. At autopsy, body, ovary and pituitary weights were obtained. The ovaries and accessory reproductive organs were fixed in Bouin's fluid and prepared for study, while the pituitaries were fixed in Regaud's fluid and stained by methods previously described.

The various weights are recorded in Table I. After 10 daily injections the ovaries of the experimental rats were increased to a mean weight of 13.4 mg., while that of the controls was only 6.1 mg. After 20 daily injections the ovaries of the experimental animals were increased to a mean weight of 62.4 mg., while the mean weight of the ovaries of the controls was 17.2 mg. These injections failed to increase the weight of the pituitaries of the experimental rats over those of the controls (Table I). This is in direct contrast to the findings when such injections are carried out in 21-day rats. Histologic examination of the ovaries of the experimental rats revealed that follicular maturation and corpus luteum formation had not occurred, but there was a marked hypertrophy of the thecal cells resulting in thecal luteinization. This process was usually well under way in the rats killed after 10 injections and was marked in the rats killed after 20 days.

Serial sections of all pituitaries were prepared for study and cell counts made. Comparison of the anterior pituitaries of the experimental rats, killed after 10 injections, with those of their controls, revealed that the level of the eosinophiles was practically the same in the 2 groups (Table I). In the controls the level of the basophiles was high and a great majority of the cells were well filled with

TABLE I.

The quantitative data pertaining to the percentages of the various cell types are given in a frequency distribution table. Class intervals are given in percentage. Mean rat, ovary and pituitary weights, as well as number of rats used, are given below.

Class Intervals %	25 U. Follutein daily—10 days		25 U. Follutein daily—20 days	
	Control	Exp.	Control	Exp.
Eosinophiles:				
30.-34.9	1	3	9	
35.-39.9	11	8	7	
40.-44.9	3	2	5	8
45.-49.9		3		4
Basophiles—Granular:				
0.-1.9		16		12
2.-3.9			1	
4.-5.9	2		2	
6.-7.9	6		8	
8.-9.9	7		10	
Basophiles—Non-granular:				
0.-1.9	12	5	9	5
2.-3.9	3	6	12	7
4.0-5.9		5		
Chromophobes:				
45.-49.9		1	3	
50.-54.9	6	4	7	
55.-59.9	9	3	11	4
60.-64.9		8		8
Total Rats per Group	15	16	21	12
Mean Rat Wt. (gm.)	29.1	28.6	50.2	54.2
Mean Ovary Wt. (mg.)	6.1	13.4	17.2	62.4
Mean Pituitary Wt. (mg.)	1.96	1.96	2.4	2.6

granules, while in the experimental rats the percentage of the basophiles was markedly decreased and those present were regressive and contained few if any granules. The chromophobes were slightly more abundant in the experimental rats. The quantitative data are given in Table I.

Comparison of the anterior pituitaries of the experimental rats, killed after 20 injections, with those of their controls shows that in the experimental animals the level of the basophiles was still very low and those present were practically devoid of granules. On the other hand, the level of these cells in the controls was much higher and a majority were well filled with granules. However, of greater interest was the high level of the eosinophiles in the injected animals of this group, which in every instance was above 40%. This was a moderate but a well defined increase over the level usually found in the controls (Table I). The eosinophiles in the anterior pituitaries of the controls and experimentals were morphologically similar, although in some of the experimental rats they were slightly larger and the negative image of the Golgi apparatus was more prominent.

7620 C

Diurnal Variation in Blood Sugar Level of the Rat.

M. CAROLINE HRUBETZ. (Introduced by H. B. Williams.)

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It has been the custom in this laboratory to begin all blood sugar experiments on the rat at 9:00 A. M. This procedure has been adopted because it has never been shown whether or not the blood sugar level of the rat varies or remains constant throughout the day. Scott[1] has shown that "when other conditions are maintained as constant as is practicable, the blood sugar in the rabbit is independent of the time of day, at least during the ordinary working hours." It is the purpose of this paper to show the relative blood sugar levels of rats throughout the day.

A total of 200 observations were made on 192 normal-fed rats. The animals were fed *ad libitum* to the time of removal from their cages for bleeding. Approximately 50 observations were made at each of the following hours: 9:00 A. M., 12:00 noon, 3:00 P. M., and 6:00 P. M. The Somogyi micro-method,[2] a modification of the Shaffer-Hartmann method,[3] was used.

The entire experiment was completed in 7 days thus reducing the probability of interference of environmental changes or of changes in the conditions of the animals. This is reflected in the constancy of the results as shown by the very small deviations. These results justify the conclusion that the blood sugar level of the rat is independent of the time of day, at least during the ordinary working hours.

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[1] Scott, E. L., *Arch. Int. Med.*, 1929, **43**, 393.

[2] Somogyi, M., *J. Biol. Chem.*, 1926, **70**, 599.

[3] Shaffer and Hartmann, *J. Biol. Chem.*, 1920, **45**, 349.

7621 C

Epinephrine and the Blood Sugar Level.

M. CAROLINE HRUBETZ. (Introduced by H. B. Williams.)

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Bang[1] presented curves for the blood sugar level of rabbits after both intravenous and subcutaneous administration of epinephrine The rise after intravenous injection was less than 100% and reached its maximum in ½ hour, that after subcutaneous injection around 300% and reached its maximum between the second and third hour after injection. One-tenth of a milligram was administered per animal.

In the present study, all injections were given subcutaneously. Abbott's "adrenalin" was diluted so that 1 cc. contained 0.4 mg., making the full dose of 0.4 mg. per kilo, or, 0.1 mg. per 250 gm. body weight. For the ½, ¼, ⅛ doses corresponding dilutions were made. The animals used were normal-fed rats. We obtain curves similar to those of Bang except that our maximum rise occurred at the 1½ hour interval after injection. This discrepancy may be explained by our larger number of observations where variations of individual animals are smoothed out, and also, our smaller dosage.

Approximately 50 observations were made at each of the intervals: 5, 15, 30, 45 minutes, 1, 1½, 2 and 4 hours after the full dose and also after the ¼ dose. In addition, 50 observations were made at the 30-minute interval for both ⅛ and ½ the full dose. Two series of controls of 50 observations each were made, the determinations of which were dispersed throughout the period of experimentation. The 0.2 cc. Somogyi modification[2] of the Shaffer-Hartmann[3] blood sugar method was used.

There is a steady rise in the blood sugar level until the 1½-hour interval is reached, the greatest change occurring during the first 20 minutes. After 1½ hours the blood sugar level is gradually reduced but does not reach the normal level in 4 hours. The mean deviations are smallest for the shortest intervals (6 to 11 mg. for 5 minutes) and become progressively greater and more variable until the 4-hour interval is reached (17 to 46 mg.). Eadie and Macleod[4] found it impossible to standardize insulin by the Epine-

[1] Bang, *Der Blutzucker*, Weisbaden, 1913, 113.

[2] Somogyi, M., *J. Biol. Chem.*, 1926, **70**, 599.

[3] Shaffer, A. P., and Hartmann, A. F., *J. Biol. Chem.*, 1920, **45**, 349.

[4] Eadie, G. S., and Macleod, J. J. R., *Am. J. Physiol.*, 1922, **46**, 285.

phrine Equivalent Method because of the great variability in the results. However, they took their blood samples at ½, 1 and 2 hours after the injection, intervals where the mean deviations are the greatest.

The blood sugar level after ⅛, ¼, ½, and the full dose ½ hour after the injection shows a steady rise in the blood sugar until the ½-dose is approached, after which the curve flattens out. Apparently, there is a maximum amount of stimulus to which the system will respond. Beyond this point, there is no increase in effects. The mean deviations vary from 13 to 29 mg., the smallest deviation occurring with the smallest dose.

Summary. 1. 1000 observations were made at 5, 15, 30, 45 minutes, 1, 1½, 2 and 4 hours after given doses of epinephrine. 2. The blood sugar reaches its highest level in 1½ hours and has not returned to normal in 4 hours. 3. With doses varying from ⅛ to the full dose, the blood sugar increases proportionately with the dosage until ½ the dose, where the curve flattens out. 4. The smallest deviations are obtained after the shortest interval after the injection, or, after the smallest dose.

7622 P

Perimetry with Stimuli of Minimal Duration.

LEO L. MAYER. (Introduced by L. J. Pollock.)

From the Departments of Ophthalmology, Nervous and Mental Disease, and Experimental Surgery of the Northwestern University Medical School.

The utility of field defects as outlined by perimetry in localizing disease along the optic pathways is fully discussed by Traquair[1] and Peter.[2] With the advent of the McHardy[3] self-registering perimeter and its electrical test objects a comparison between white and colored lights became available.

During the course of some experiments upon the chronaxia of the optic nerves projected by Davis and Pollock, a method of perimetry with a light stimulus of very short duration has been developed.

[1] Traquair, H. M., Introduction to Clinical Perimetry, American edition, St. Louis, C. V. Mosby Co., 1927.

[2] Peter, L. C., Principles and Practices of Perimetry, 2nd Edition, Philadelphia, Lea and Febiger, 1923.

[3] McHardy, M., *Ophth. Rev.*, 1882, **1**, 107.

Our immediate problem was to compare the fields obtained by using a test object of varying size and color at infinite duration with that of a flash of light of a known and standard candle power and varying extremely short duration. Thus not only energy but time was considered a factor in our work. As the peripheral portion of the retina is stimulated there is a distinct varying limit to the recognition of light flashes of short duration, the more peripheralwards the stimulus, the longer must be its duration to be perceived. This has been amply confirmed by a previous study.[4] The work of Sheard[5] would seem to show that such a minute quantity as a quantum of light is enough to stimulate the fovea.

The instrument for producing such short flashes consists of a small neon bulb which is activated by condensor discharges through the use of "B" batteries. Flashes of the speed of 1/25,000 of a second were produced for this initial experiment. The neon bulb was blackened so that the light emitted occupied an area comparable to the ordinary 2 mm. test object, and the bulb was mounted on a hand perimeter where it would be freely movable. A norm was established by recording the averages of 3 determinations on the eyes of each of 20 normal individuals. As the neon light is red in color it was necessary to determine whether the color itself was a factor. It was definitely shown that the field with the neon lamp did not correspond with the ordinary field for red.

The cases studied have been grouped as follows: (1) Normal fields in suspected intracranial disease, (2) Fields in hysteria, (3) Fields in pituitary disease, (4) Fields in other intra-cranial lesions, and (5) Fields in intraocular disease.

In the first group it was shown that the flash field afforded a more accurate indication than a form field or at times other localizing signs. For example, in a patient with X-ray evidence of *sella turcica* destruction a normal flash field being found, operation failed to reveal any pathology interfering with the optic pathways. Ten cases were included in this group. In spite of the fact that ordinary perimetry with a white target was suggestive of a characteristic field defect in these cases, the examination by the flash method revealed normal fields. Subsequent examination of these cases confirmed the findings of the normal flash field.

The outstanding observation in the group of fields in hysteria was the fact that in *no* case was a tubular field demonstrated by the flash method although ordinary perimetry had shown this defect.

[4] Mayer, L. L., *Arch. Ophth.*, 1933, **9**, 353.

[5] Sheard, C., *Am. J. Physiol. Optics*, 1922, **3**, 126.

Twenty cases were in this group. Most of the patients were epileptics in whom an organic basis was suspected.

In a large group with pituitary disease the flash method more critically emphasized the field defect. For example, where the ordinary perimetric study revealed a temporal field including the ninetieth meridian, the flash method recorded this temporal field to only the fiftieth meridian. Even meticulous studies with the Bjerrum method[6] did not approximate the accuracy shown by the flash method. Detailed studies of the 28 patients of this group will be published later.

Group 4 in which other intracranial lesions were investigated consists of a heterogeneous group with various lesions. Here the outstanding features of the flash method were the ease with which the patient was able to determine the critical point, the definiteness of the test target, and certain deviations from the usual findings in ordinary perimetry, such as bizarre configurations of the fields, etc. Six patients in this group demonstrated the dependability of the flash method in comparison with ordinary perimetry. Definite and characteristic field defects were demonstrable earlier and more accurately.

Of the last group in which intraocular disease was investigated, it appears that a closer check on the reduction of the field due to glaucoma may be had by this method. Also a better method of prognosticating in operated cases of detached retina is at hand. Only 2 cases of retinal detachment and 2 of glaucoma are included in this group as the study of ocular conditions, *per se,* has just started.

Many of the patients examined by this method have been operated, others have been autopsied, and confirmation of the flash findings were made. These will be reported later.

Conclusions. 1. A method is described in which a rapid light flash is made use of as a perimetric target. 2. The technique of the examination is simple, neither the observer nor the patient become fatigued by the procedure and the perception of the flash being critical. The flash is recognized or it is not seen. Field defects are discovered to a degree not detectable by the older, far more painstaking method, which required greater cooperation and intelligence on the part of the patient. 3. Although a complete interpretation of all the findings delineated by this method must await further case study and subsequent confirmation by biopsied and autopsied materials, it appears that the method has certain advantages in localization of lesions of the optic pathways.

[6] Bjerrum, *Verhandl. d. X Internat. Med. Cong.,* Berlin, 1890, 466, (II).

7623 C

Protein-Free Suspensions of Virus: VI. Purification of Vaccine Virus by Adsorption and Elution.

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It was shown[1] that by repeated elution with N/500 $NH_4(OH)$ from the same adsorbate it was possible to obtain a potent phage suspension which gave negative protein and ninhydrin tests and contained only 1.4 to 2.0 mg. non-ammonia N per 100 cc. of eluate.

In the present paper experiments are reported showing that it is possible with the same procedure to obtain an equally pure active suspension of vaccine virus.

Technique. An infected rabbit testicle is removed on the 4th or 5th day after inoculation, ground in a mortar with sterile glass, and saline added slowly to give a 10% tissue suspension. After thorough trituration the tissue suspension is transferred to a centrifuge tube and centrifuged slightly to remove coarser particles. The material is handled aseptically to avoid contamination during the preparation. The heavy tissue suspension is then added to tubes containing 50% kaolin in saline, in the proportion of one part virus to one part kaolin suspension, thoroughly shaken, left in the icebox overnight and then treated in the manner previously described.[2]

The following experiments illustrate the details of the procedure employed and results obtained:

Experiment 1. The suspension was prepared in saline and adsorbed in buffered and unbuffered saline suspensions of kaolin. The adsorption was carried out in duplicate and each adsorbate eluted separately. The supernatant fluids after the kaolin adsorption as well as the individual eluates from the adsorbate were tested on rabbits by the injection of 0.1 cc. of the material intradermally. The results are summarized in Table I.

Adsorption with kaolin removed the greater part of the protein and virus from the suspension, and repeated elution with ammonia ultimately yielded a potent virus suspension which gave a negative protein reaction. The buffered kaolin adsorbed more completely but showed no difference in its response to elution. The first and second eluates still gave positive tests for protein and aminoacids; the third eluate, however, no longer gave positive Esbach and ninhydrin reac-

[1] Kligler, I. J., and Olitzki, L., *Brit. J. Exp. Path.*, 1934, **15**, 14.

Protocol. One gm. testis triturated and suspended in 10 cc. saline. After slight centrifugation, 8.0 cc. of the uniform suspension were used for adsorption. Adsorption with buffered (pH 5.6) and unbuffered kaolin; left in icebox 24 hours. The contents of each of the 2 tubes were eluted with: (I) 4 cc. N/100 NH_4OH, or (II) 4 cc. N/500 NH_4OH. Rabbits inoculated 11/23/33. Reactions recorded as —, +, ++, +++, according to area of induration.

TABLE I.

	Rabbit reaction*	Esbach	Ninhydrin
Supernatant fluid after adsorption:			
(a) with buffer I	+	+	+++
(b) " " II	±	+	+++
(c) without buffer I	+	+	+++
(d) " " II	++	+	+++
Eluate I:			
with buffer N/100 NH_4OH	+	+	+++
" " N/500 "	+	+	+++
without buffer N/100 NH_4OH	++	+	+++
" " N/500 "	++	+	+++
Eluate II:			
with buffer N/100 NH_4OH	+++	±	++
" " N/500 "	+++	±	++
without buffer N/100 NH_4OH	+++	±	±
" " N/500 "	++	±	±
Eluate III:			
with buffer N/100 NH_4OH	++	—	—
" " N/500 "	++	—	—
without buffer N/100 NH_4OH	++	—	—
" " N/500 "	++	—	—

*Reading 4th day after inoculation.

tions, although it still contained active virus. The second eluate contained the highest concentration of virus.

Experiment 2. This experiment was designed to ascertain the amount of virus eluted and the conditions most favorable for maximum elution. The original material as well as the respective eluates were titrated on the rabbit skin. To overcome the error due to variations in susceptibility of individual rabbits, a given dilution of each of the materials was inoculated into the same animal. Each animal was thus inoculated on 6 separate points, 5 rabbits being used for the experiment. This procedure also facilitated the comparison of the relative intensity of the reactions to different eluates, making the results as nearly quantitatively comparable as is possible in animal work. Nitrogen determinations were made on all the materials tested.*

It will be noted that the kaolin adsorbs about 80% of the protein and nearly all the virus. It is also of interest that the first eluate contains most protein and is weakest in virus; this is probably due to the neutralization of the NH_4OH by the buffer, thus

*The nitrogen determinations were made by Dr. Rosenberg.

Protocol, Exp. 2. Material not centrifuged before adsorption. Tissue emulsion 1/10 mixed with equal amounts of kaolin suspension. All elutions made with N/500 NH_4OH. B = with buffer; W = without buffer.

TABLE II.

	Original material	Supernatant fluid after adsorption		Eluates (undiluted)							
				I		II		III		IV	
		B	W	B	W	B	W	B	W	B	W
(a) Test of Virus.											
Rabbit Test*	+++	++	++	++	+++	+++	+++	+++	+++	++	++
Non NH_3 N (mg. %)	280	45.8	33.4	5.12	9.1	4.72	6.31	3.95	5.44	2.68	1.04
Esbach Reaction	+++	++	++	+	+	±	±	tr.	tr.	—	—
Ninhydrin Reaction	+++	++	++	+	+	±	±	±	±	—	—

(b) Titration of Virus.

	Dilution tested	Original suspension	Supernatant Fluid	Eluates I	Eluates II	Eluates III	Eluates IV
Rabbit 1	1:100	+++	±	+	+	+	±
" 2	1:1,000	++	±	+	++	++	++
" 3	1:10,000	+++	—	±	+	++	++
" 4	1:100,000	++	—	±	+	+	±
" 5	1:1,000,000	+	—	—	±	+	±

*—, +, ++, +++ indicate relative intensity of the reaction. The rabbit results are the readings on the 5th day after inoculation.

reducing its eluting capacity. The third eluate was the most potent, but the fourth eluate was still active in a 1:1,000,000 dilution, the highest dilution tested. The protocol also brings out clearly the differences in the reactivity of animals, the 1:1,000 dilutions producing milder lesions than the 1:10,000.

Experiment 3. The previous experiment was repeated with the purpose of carrying the dilutions to the maximum and comparing the virus content in the respective eluates with that in the original suspension. The procedure was the same as before. The results are summarized in Table III.

The titration experiments yielded paradoxical results. The eluates were active in as high a dilution as the original tissue emulsion; at the same time, in the lower dilutions, the reactions produced by the tissue suspension were more severe than those produced by the eluates. The greater severity of reaction produced by the original tissue suspension is ascribable to the phenomenon observed by Duran-Reynals.[2] The testicular extract increases the invasiveness

[2] Duran-Reynals, F., *J. Exp. Med.*, 1929, **50**, 327.

Protocol. Infected testes were triturated and a 10% suspension made in saline. The adsorption was made as above by mixing equal portions of the suspension with buffered kaolin. The elution was carried out with N/200 NH_4OH. Four successive elutions were made. The eluates as well as the original suspension were titrated on the rabbit skin. The readings were made on the 4th and 5th day after inoculation. The table gives the results at the last reading.

TABLE III.

Virus Dilutions	Original Suspension	Eluates			
		I	II	III	IV
10,000	+++	++	++	++	++
100,000	+++	+	++	++	++
1,000,000	+++	+	+	±	++
10,000,000	±	±	±	±	++
Esbach reaction	+++	+	+	tr.	—
Ninhydrin	+++	++	+	±	—

The signs ±, +, ++, etc., indicate intensity of reaction; tr. = trace.

of virus by increasing tissue permeability, thus causing a more extensive lesion. Normal testicular extract added to the eluates increases the extent and severity of the lesion.

Summary. Experiments are presented showing that it is possible by adsorption with kaolin and subsequent successive elutions with ammonia to obtain a potent suspension of vaccinia virus giving negative Esbach and ninhydrin tests and containing 1.0 to 2.7 mg. non-ammonia *N* per 100 cc. of fluid. The severity of the reaction produced by a given dilution of the original testicular suspension is always greater than that produced by an equal volume of the same dilution of the eluted pure virus. In most instances the eluates were active in as high a dilution as the original suspension. The vaccinating efficiency of the pure virus has not yet been tested, but it is anticipated that it may prove useful for intradermal vaccination, particularly because of the milder character of the reaction produced by the purified virus.

7624 C

Observations on Cellular Oxidative Mechanisms Involved in Dinitrophenol Stimulation of Respiration.

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In an extensive investigation of the effect of dinitro compounds on tissue respiration and cell division, a point has been reached where it appears desirable to know more about the mechanism by which such compounds stimulate oxidative processes in the cell. We report here some experiments with 4,6 dinitro-o-cresol (DNC)* which indicate that dinitro compounds do not act on cell respiration either in the same way as methylene blue and other dyes which are reduced by the cell and re-oxidized by molecular oxygen,[1] or in the same way as dimethyl-p-phenylene diamine, which is reduced by the cell and reoxidized by the indophenol oxidase.[2]

When the oxygen consumption of sea urchin eggs is raised by DNC to 400% of the normal,[3] the R.Q. remains unchanged at the normal value of about 0.93. We have found that DNC is not an autoxidizable catalyst for the oxidation of glucose or cysteine, even in the presence of traces of metals or cytolyzed animal tissue. We have also found with rat tissues and eggs of invertebrate marine animals that the action of DNC as a respiratory stimulant can be completely and reversibly blocked by cyanide. Field, Martin and Field report similar results with cyanide on yeast.[4]

These facts indicate that DNC acts upon one or more of the cyanide sensitive oxidative chains in the cell. These may be roughly divided into 2 classes: (A) those depending for oxygen activation on Keilin's cyanide sensitive indophenol oxidase, and (B) those in which the substrate is activated by an autoxidizable cyanide sensitive dehydrase.[5] The mechanisms classifiable under (B) do not act through cytochrome, hence a distinction between the 2 classes can be made by studying the effect of DNC on the rate of oxidation or reduction of cytochrome in respiring cells.

* This compound is 1-methyl 2-hydroxy 3,5 dinitro benzene.

[1] Barron, E. S. G., *J. Biol. Chem.*, 1929, **81**, 445.

[2] Keilin, D., *Proc. Roy. Soc.* (London), B, 1929, **104**, 206; Runnström, J., *Protoplasma*, 1932, **15**, 532.

[3] Krahl, M. E., and Clowes, G. H. A., *Biol. Bull.*, 1934, **67**, 332.

[4] Field, J., 2nd, Martin, A. W., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 997.

[5] Dixon, M., *Biol. Rev.*, 1929, **4**, 352.

A preliminary rough experiment, made August 20th, 1934, on a 25% suspension of normal yeast showed that the time of reduction of cytochrome was greatly accelerated by DNC, the time required, after aeration, for reappearance of the d band being reduced from 27 seconds to 5 seconds. After repeated confirmation of these results experiments were conducted on starved yeast with DNC in the presence of a variety of substrates.

Typical results of such experiments on starved yeast are presented in Tables I and II. The procedure in each case was as follows: A 3 cc. portion of a well aerated 17% suspension of Fleischmann's yeast in pH 6 McIlvaine phosphate-citrate buffer was mixed with 1 cc. portions of the designated substrate, DNC, and buffer solutions to give a constant volume of 5 cc. The final concentrations were: Yeast, 10%; substrate, 0.2%; DNC, 5×10^{-6}M for the experiments in Table I, and 6.25×10^{-5}M for the experiments in Table II. After 10 minutes reaeration, the effect was registered by noting the number of seconds which elapsed prior to the reappearance of the strong d band. Since the degree of acceleration effected by the DNC varies according to the length of time that the suspension has been allowed to stand, controls with yeast and buffer alone and yeast and DNC and buffer were run at frequent intervals in the course of the experiments.

TABLE I.

	Cysteine		Succinate		Glucose		Pyruvate		Lactate	
	None	0.2%	None	0.2%	None	0.2%	None	0.2%	None	0.2%
No DNC	292	272	285	243	223	63	164	65	106	93
5×10^{-6}M DNC	66	58	62	53	63	27	61	56	45	49

TABLE II.

	Cysteine		Succinate		Glucose		Pyruvate		Lactate	
	None	0.2%	None	0.2%	None	0.2%	None	0.2%	None	0.2%
No DNC	422	303	346	297	254	89	194	104	118	96
6.25×10^{-5}M DNC	90	85	89	100	91	50	88	94	86	83

The data show (a) that DNC greatly accelerates the reduction of cytochrome, and (b) that only with glucose is the reducing ability of the combination equal to the sum of those of the individual agents. Indeed, the pyruvate plus DNC and lactate plus DNC are no more efficient than DNC alone, although pyruvate and lactate both have a large reducing effect when used singly. It may be noted that this inability of lactate to increase the reduction of cytochrome in the

presence of DNC may account for the aerobic glycolysis which dinitro compounds produce in tissues.[6]

In subsequent experiments with iodoacetate poisoned yeast, carried out in the manner described above with M/1500 iodoacetate in the pH 6 buffer, it was found that DNC gave no acceleration of cytochrome reduction even when lactate or glucose was present. Since others[7] have observed that iodoacetate blocks the acceleration by 2,4 dinitro phenol of respiration in yeast, it is likely that some sulfhydryl containing enzyme system is essential for the DNC action.[8]

We have also found that such non-specific dehydrase poisons as sodium pyrophosphate and narcotics inhibit to a limited degree the reactivity of tissues to DNC stimulation.

From the evidence available we believe it likely that DNC stimulates cellular respiration by accelerating the oxidation by cytochrome of some substrate previously or simultaneously acted upon by the anaerobic dehydrases of the cell. It is too early to say whether DNC acts as a diffusible oxygen carrier between cytochrome and the substances normally oxidized or as an artificial substitute for a co-enzyme in the activation of substrates which do not normally play an important rôle in respiration. We are continuing these studies with cell-free cytochrome and individual dehydrases in an effort to demonstrate the relation between the individual components of such a system, eliminating at the same time the complicating factors connected with variations in the permeability of the cell.

7625

Studies on Acholic Cachexia: IV. Relation of Biliary Diversion to Duodenal Ulcer Formation.*

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The occurrence of peptic ulcer of the duodenum after the exclusion of bile has been noted with a wide variation in frequency and the factors concerned are not clearly understood. Kapsinow and

[6] Dodds, E. C., and Greville, G. D., *Nature*, 1933, **132**, 966; Dodds, E. C., and Greville, G. D., *Lancet*, 1934, **1**, 398.

[7] Ehrenfest, E., and Ronzoni, E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 318.

[8] Dickens, F., *Biochem. J.*, 1933, **29**, 1141; Michaelis, L., and Schubert, M. P., *J. Biol. Chem.*, 1934, **106**, 331.

*Work done in part under a grant from the Jessie Horton Koessler Fellowship.

Harvey,[1] using a cholecystnephrostomy, produced ulcer in 17 out of 43 animals. Bollman and Mann[2] by simply ligating the common ducts produced ulceration in about 60%. In the experiments of Berg and Jobling[3] in which a Rous type of fistula was used, ulcers resulted in 13 of 23. Kim and Ivy report 10% ulcers in one series[4] in which the common duct was tied, but 60% in cases of Rous fistula.

Besides the obvious acid factor, there is considerable evidence that other influences are at work. Ivy has suggested that the mechanical irritation of the tubing near the duodenum may be important. On the other hand Elman has seen but few ulcers resulting from bile fistula alone. Our own experience has been that simple duct ligature alone caused ulceration in numbers about one-half the rate usually suggested (60%), but that a very high percentage resulted from the Rous fistula which has proven very difficult technically in our hands. Berg suggests that the general condition of the dog is very important and found that animals kept under poor hygienic conditions developed ulcer in 100% while those better cared for showed only 30%.

Using the Dragstedt cannula for making the biliary fistulas we have been surprised at the freedom from this complication. Twenty such experiments have been made and not a single ulcer has developed, as evidenced either by post-mortem examination or presence of blood in the stools. These animals have been fed on various diets, some with large amounts of meat and others with high carbohydrate content. Some have undergone periods of starvation and several had one or more exploratory laparotomies. All had total fistulas in which no licking of bile occurred and all lost weight progressively. Several had lost as much as a third of body weight. Some lived over 5 months.

These experiences suggest that the general condition of the animal is not the deciding factor. It would be hard to find animals more cachetic than ours. Diet is evidently not important. In view of the fact that previously our bile fistula dogs prepared by cholecystnephrostomy or by Rous fistulas constantly developed ulcers, and that the incidence of ulcer among duct ligature dogs was high, it seems suggestive that the only common factor is concomitant liver damage. In all the above types of operations, in our hands at least, there has been a progressive development of degenerative and in-

1 Kapsinow, R., Eagle, L. P., and Harvey, S. C., *S. G. O.*, 1924, **39**, 65.

2 Bollman, I., and Mann, F. C., *Arch. Surg.*, 1932, **24**, 126.

3 Berg, B. N., and Jobling, J. W., *Arch. Surg.*, 1930, **20**, 997.

4 Ivy, A. C., and Fauley, L. N., *Am. J. Surg.*, 1931, **11**, 531.

fective changes in the liver parenchyma. In the successful type of Rous fistula (ours were usually failures) Elman found very few ulcers. Berg's experience was similar. His smoothly functioning Rous fistulas developed many ulcers while the unsuccessful ones developed many.

It appears, therefore, that the development of duodenal ulcer in the absence of bile from the intestine is partially dependent on some factor other than acid. Possibly the effect on the gastric motility of liver damage, as suggested by Still and Carlson,[7] is the deciding factor.

7626 C

Electrocardiographic Studies of Chemical Pericardial Irritation.

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From the Lasker Foundation for Medical Research and the Department of Medicine of the University of Chicago.

Pericardial effusions, clinical or experimental, may result in electrocardiographic changes much like those of coronary occlusion or of ligation of the coronary arteries.[1–7] In experimental effusions, one must consider the effect of pressure and of possible chemical influence. Indeed, Wiggers[8] has demonstrated bizarre ventricular complexes, similar to those of effusions, resulting from applying a few cubic centimeters of potassium chloride solution to the visceral pericardium. Hence it seemed desirable to study the effects of various bland and irritating chemicals under conditions to exclude any pressure factor.

Dogs were used, with preliminary morphine sulphate 0.015 gm.

5 Kim, M. S., and Ivy, A. C., *J. A. M. A.*, 1931, **47**, 1511.

6 Elman, R., and Hartmann, A. F., *Arch. Surg.*, 1931, **23**, 1030.

7 Still, K. S., and Carlson, A. J., *Am. J. Phys.*, 1929, **89**, 34.

* I am deeply indebted to Dr. Louis Leiter and Dr. Emmet Bay for advice during this work.

1 Smith, F., *Arch. Int. Med.*, 1918, **22**, 8.

2 Smith, F., *Arch. Int. Med.*, 1920, **25**, 673.

3 Smith, F., *Arch. Int. Med.*, 1923, **32**, 497.

4 Barnes, A. R., and Mann, F. C., *Am. Heart J.*, 1932, **7**, 477.

5 Scott, R., Feil, H., and Katz, L., *Am. Heart J.*, 1929, **5**, 68, 77.

6 Bay, E. B., Gordon, W., Adams, W., *Am. Heart J.* 1933, **8**, 525.

7 Harvey, J., and Scott, J. W., *Am. Heart J.*, 1932, **7**, 532.

8 Wiggers, C., *Am. Heart J.*, 1930, **5**, 346.

about one-half hour before the first or control electrocardiogram. Light ether anesthesia followed, then rib resection just to the left of the sternum to expose the pericardium, followed by artificial respiration and opening the pericardial sac by a 3 to 5 cm. anterior incision. There was an electrocardiographic control at each step. The pericardium and chest were not closed again.

The chemicals used were liquids, solutions, and solids (crystals, powders), the fluids in amounts averaging one cubic centimeter applied at body temperature with a medicine dropper or camel's hair brush, so that pressure effects were absent. The solids were dropped on lightly. The initial application was made over the left ventricle just to the left of the septum and above the apex. Subsequent spreading of fluids was unavoidable.

The experiment was stopped if the dog went into shock, became asphyxiated, or developed ventricular fibrillation.

Electrocardiographic Results. Controls. These proved to be very important. The ST takeoff varied from +2 mm. to —1 mm., and in lead II half the T waves were negative or diphasic. Ether anesthesia caused surprising transient voltage decrease, as well as rate increase, and opening the pericardial cavity to atmospheric pressure and room temperature caused another voltage decrease (all leads) which did not tend to return to the control level.

Furthermore, the control ST segment was decidedly convex or concave in some cases; and about half the time, ST or T changed direction in at least one lead after ether anesthesia.

TABLE I.
Substances Producing ST Elevation as the Chief Result.

Alcohol (95%)	ST rises starting in 12 seconds, with return to normal in 1 minute. An even greater rise occurred with reapplication over an artery.
Calcium Chloride (7%-10% sol.)	Marked ST rises within one-half minute, maximal in 3 minutes, then partial return to normal. (Concentrations of less than 7% were ineffective.)
Ether (U.S.P.)	Marked sudden ST rise with sharp T inversion.
Ethyl Chloride (U.S.P.)	ST elevation and deep T waves.
Formic Acid (U.S.P.)	ST rise in 10 seconds, starting towards normal in 1 minute.
Hydrogen Peroxide (U.S.P.)	Transient ST and T change more marked when application was over an artery. Voltage decrease.
Lactic Acid (1%)	Instantaneous ST elevation.
" " (Conc.)	Rapid but not instantaneous ST elevation.
Mercurochrome (Crystals)	ST elevations starting in 6 minutes, maximum in 15 minutes.
Potassium Chloride (1%-10% sol.)	ST rises similar to those from calcium chloride, and agreeing with Wigger's results.[8]
Sodium Chloride (Crystals)	Moderate ST elevation.
Tincture of Iodine (U.S.P.)	Similar to alcohol. (Tincture of iodine contains 83% alcohol.)

In a few instances, for control purposes, nothing was done for several hours after opening the pericardium; there were no additional electrocardiographic variations. In about half the experiments, the chemical was applied first to the intact parietal pericardium; there was never any significant change. Incidentally, lead II alone usually gave all the necessary information.

Application to the Visceral Pericardium. The most frequent change was a genuinely significant rise in the ST interval, amounting at times to RT fusion, and substances quite different chemically produced similar results. (Table I and Plate I.) Complete bundle-branch block occurred 3 times, in each case with similar substances. (Table II.)

There was a group giving miscellaneous results. (Table III.) The time of appearance of the changes varied from instantaneous

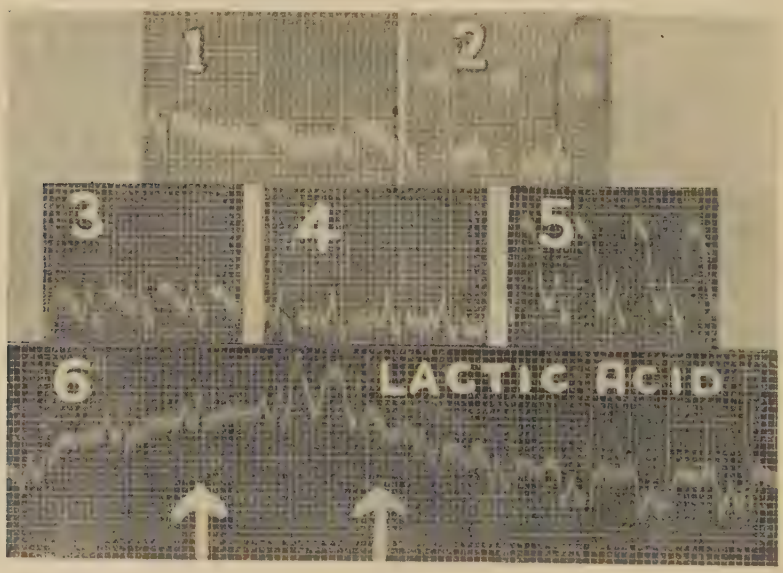

PLATE I.
(Lead II throughout).

FIG. 1-2. Calcium Chloride.
Fig. 1—30 seconds after applying 7% calcium chloride sol.
Fig. 2—4 minutes " " " " " "

FIG. 3-5. Alcohol.
Fig. 3—12 seconds after applying 95% alcohol to the muscle.
Fig. 4—60 " " " " " " "
Note return toward normal.
Fig. 5— 5 seconds after re-application over branch of left coronary artery.

FIG. 6. Lactic Acid.
Fig. 6—Arrows indicate moment of application of lactic acid, 1% sol.
Note the immediate ST plateau-like rise.

TABLE II.
Substances Producing Complete Bundle Branch Block.

Oil of Cloves (U.S.P.)	Complete in 1 hour.
Sandalwood Oil (U.S.P.)	" " 28 seconds.
Turpentine (U.S.P.)	" " 9 minutes.

(In each of these instances, the PR interval was unaltered, the evolution of the block was gradual, with no transitional period resembling arborization block.)

TABLE III.
Substances Producing Miscellaneous Results.

Ammonia Water (1%-10% U.S.P.)	Occasional extrasystoles and slight ST changes.
Barium Chloride (1.5% sol.)	Varying runs of extrasystoles.
Chloroform (U.S.P.)	Sudden, sharp, diphasic T wave.
Potassium Permanganate (0.1% sol.)	Slight QRS notching, diphasic T, and high P.
Silver Nitrate (20% sol.)	Temporary QRS widening, but after bilateral vagotomy extrasystoles occurred, and after atropine, nothing happened, except the slower rate.
Sodium Carbonate (U.S.P.)	Transient ventricular tachycardia of tremendous voltage. Later, ST and T high. Still later, return to normal.
Sodium Hydroxide (10% sol.)	Bizarre notchings of uncertain significance. (Possibly respiratory variations.)

(see lactic acid record on Plate 1) to an hour or more. The changes with alcohol, calcium chloride solution, lactic acid, formic acid, sandalwood oil, and sodium carbonate crystals were some of those occurring within one minute.

It is interesting to note the variety of substances that were without significant effect. (Table IV.)

TABLE IV.
Substances Producing No Significant Result.

Acetone (U.S.P.)	Normal Saline
Chrysarobin (crystals)	Potassium Chlorate (5% sol.)
Ephedrin Sulphate (3% sol.)	Talc (U.S.P.)
Glucose (U.S.P.)	Tannic Acid (5% sol.)
Lactose (U.S.P.)	Xylol (C.P.)

End Effects. Some of the changes returned toward normal; others progressed into ventricular fibrillation, or were not observed. After several hours, it was sometimes difficult to tell whether the effects observed were due to the original application, shock, or asphyxia.

Preliminary Atropinization or Bilateral Vagotomy. These procedures gave important variations from the usual results in some cases. (The average dose of atropine was 0.36 mg. per kilo intravenously). Thus, alcohol caused a marked ST rise which failed to occur after atropine. Silver nitrate, which caused temporary widening of the QRS, resulted in extrasystoles after bilateral vagot-

omy, and in no change at all after preliminary atropine. However, the typical calcium chloride results were not prevented by atropine. Unfortunately, the experiments in which the volatile oils (with their bundle branch block type of curve) were used after preliminary atropine, gave unreliable results.

Postmortem Observations. Thirty-four hearts were autopsied. None showed gross myocardial lesions, nor gross pericarditis. Microscopic sections were made under and adjacent to the irritated areas in 9 dogs (in the experiments using formic acid, hydrogen peroxide, potassium permanganate sol., sandalwood oil, sodium hydroxide sol., turpentine, ammonia water, mercurochrome crystals, and tincture of iodine). In the last 3 of these, fragmentation and loss of striation were taken as evidence of a muscle injury to a depth of 0.1 to 0.3 mm. Otherwise there was no apparent muscle damage; pericardial injury was variable but slight.

Significance of the Changes. Can one explain the chief changes, ST elevation of various types, and 3 instances of bundle branch block? The experiments do not furnish proof, but they do show that demonstrable muscle injury need not be present to produce the changes described, and suggest that the extracardiac nerves may be a factor. Of course, the failure to find histologic evidence of muscle injury does not preclude the possibility that the observed effect of certain substances (*e. g.,* salts) depends upon absorption, and passage of ions between muscle fibers. Moreover, bundle branch block need not depend upon absorption. There have been fairly numerous reports of transient bundle branch block, wherein there was held to be no heart damage, with increased vagal tone the factor responsible.[9–13]

The question of local or transient ischemia is not within this discussion. It is certainly questionable that any ischemia occurred in the experiments here reported. We note that alcohol or hydrogen peroxide applied over a coronary artery gave results more marked than over the muscle alone. One possibility is that the arterial application produced constriction and ischemia, but it is just as reasonable to assume increased reflex effects if the nerve supply is richer along the arterial course.

Summary. The electrocardiographic results of applying various

[9] Wolff, L., Parkinson, J., and White, P. D., *Am. Heart J.*, 1930, **5**, 685.

[10] Faulkner, J. M., *Med. Clin. of N. Am.*, 1932, **15**, 997.

[11] Newman, M., *Br. Med. J.*, 1931, **2**, 1134.

[12] Carr, F. B., *New Eng. J. Med.*, 1933, **209**, 1101.

[13] Morris, R. S., and McGuire, J., *Am. J. Med. Sci.*, 1932, **184**, 202.

bland and irritating chemicals to the visceral pericardium, with pressure factors absent, are described. The chief of these are marked ST elevations of several types, occurring quickly; bundle branch block; and the rarity of extrasystoles. The controls proved very important, and are emphasized.

7627 C

Effect of Maximal Feeding on Metamorphosis in Amblystoma.

FRANCES DORRIS. (Introduced by R. G. Harrison.)

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Twitty and Schwind[1] have shown that in heteroplastic grafting between the 2 species *Amblystoma tigrinum* and *A. punctatum,* comparable results with respect to growth are obtained only when the hosts are all maintained at the same nutritional level, an effect obtained by maximal feeding, thus insuring the rapidly growing voracius tigrinum larvae opportunity to realize their full capacity for growth. The effect of maximal feeding upon the more slowly growing species has not been emphasized, although various workers have noted that along with the spectacular acceleration of the growth rate, and a consequently earlier metamorphosis, there is a high mortality during the metamorphic period in maximally fed groups of *A. punctatum,* regardless of the type of diet.

The present experiments were undertaken in order to show the results of quantitative variation in a single diet upon the developmental rate and viability of *A. punctatum* from the earliest feeding stage through metamorphosis. Two hundred animals, taken from several bunches of eggs all at approximately the same stage of development, were reared from stage 39 onward in separate finger bowls. At the feeding stage the animals were divided into 4 groups of 50 each. The first 50 animals were starved, the second group given one feeding weekly, the third fed 3 times a week, and the fourth fed maximally. Enchytraeus, a small white worm, was used as the sole diet. All animals were measured at approximately 2-week intervals, the total length being recorded.

Fig. 1 shows the average growth rate for each group. The minimally fed larvae ate poorly, had low vitality, and remained of ap-

[1] Twitty, V. C., and Schwind, J. L., *J. Exp. Zool.*, 1931, **59**, 61; Twitty, V. C., and Elliott, H. A., *J. Exp. Zool.*, 1934, **68**, 247.

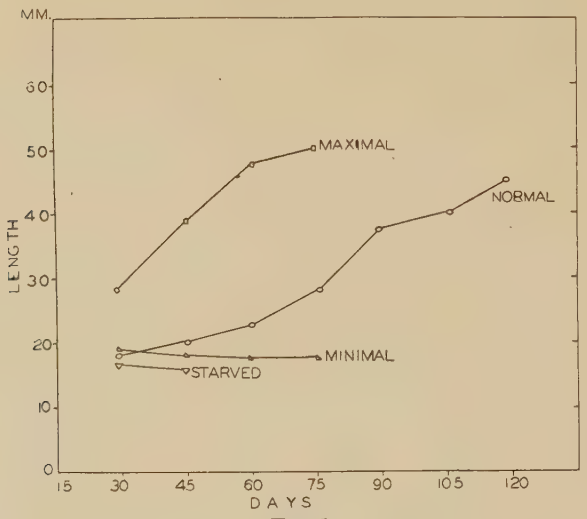

FIG. 1.
Growth rate in *A. punctatum.*

proximately the same size. By the 75th day most of these animals had died, before the 90th day the group was extinct. However, these animals survived a month longer than the starved group, all of which died by the 45th day. Three feedings a week—marked as "normal" because they approximate the usual laboratory feeding schedule—were enough to maintain a growth rate roughly about one-half that of the maximally fed group. The growth of the latter group approaches that of Twitty's maximally fed animals in both rapidity and ultimate size attained; the largest specimen was 57 millimeters long by the 60th day, as compared with 46 millimeters reached by the largest normally fed animal by the 120th day. The average final size of the maximally fed animals is no greater than that reached by the same species in nature (Dempster[2]) but the growth rate is much more rapid and metamorphosis is earlier than in the free-living specimens.

Correlated with the marked acceleration of the growth rate by maximal feeding, was a high death rate, particularly in late larval stages. Early larval deaths were apparently no more frequent in one group than in another. In Fig. 2 the percentage of survivors is plotted against time in days, and the average body length in millimeters is indicated in small figures for the maximally and normally fed groups. The high mortality in the maximally fed group began after the animals had reached an average length of 40 millimeters, with only 15% surviving by the time the group average

[2] Dempster, W. T., *Biol. Bull.*, 1930, **57**, 182.

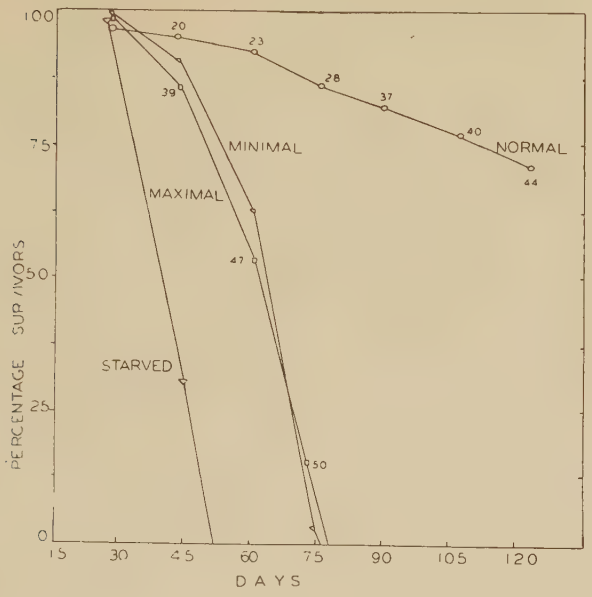

FIG. 2.
Death rate in *A. punctatum*.

had reached 50 millimeters. This is the length at which metamorphosis usually occurs, and 35% of the animals dying at this time were almost completely metamorphosed, with the gills reduced to short stumps and the color pattern changed. No maximally fed animals were brought through metamorphosis, although in the normal group over 50% of the animals metamorphosed normally at about the 120th day.

This high mortality at metamorphosis in maximally fed *A. punctatum* is in complete agreement with the work of Twitty (personal communication) who has used this method of feeding for several seasons and with larvae from different localities. It may also explain the failure of Patch[3] to obtain metamorphosis in *A. punctatum* fed on Enchytraeus. These results indicate that maximal feeding is not practicable in this species except in the earlier larval stages.

[3] Patch, E. M., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 218.

7628 C

Mechanism of the Emetic Action of the Chaulmoogrates.*

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From the Pharmacological Laboratory, University of California Medical School, San Francisco.

Read[1] recently has cast doubt upon the finality of evidence previously advanced by himself[2] and others[3] as to the central emetic action of chaulmoogrates. The possibility of sufficient salivary secretion of chaulmoogrates to bring about local gastro-enteric irritation and reflex emesis negates conclusions drawn from observed effects of parenterally administered chaulmoogrates. It thus appears desirable to establish by criteria other than taste alone whether or not chaulmoogrates are present in saliva following therapy and also to study the nature of the emetic effect further.

Absence of rapid racemization of d-chaulmoogric acid in the body has been shown by Walker, MacArthur and Sweeney.[4] In the present experiments no traces of d-chaulmoogric acid were found polariscopically in ether-alcohol extracts of 5 to 10 cc. acidified saliva samples of dogs, cats and a human volunteer taken after oral administration of gelatine capsules of chaulmoogric acid in doses of 10 to 100 mg./kg. as the strongly dextrorotatory Na salt or ethyl ester. Concentrations to be effective *in vivo* in producing emesis would presumably exceed the 0.001 to 0.01 M solutions of Na chaulmoograte we have found active in stimulating isolated rabbit duodenum. Assuming a daily flow of 1400 cc. of saliva for a human, approximately one-fifth of the administered dose of 10 mg./kg. must then be excreted by the salivary glands to maintain a concentration of 0.01 M Na chaulmoograte in the saliva for one hour, which in itself appears improbable. Further, it is generally accepted

* Part of a coöperative study of the chemotherapy of leprosy conducted by the Pacific Institute of Tropical Medicine within the Hooper Foundation for Medical Research, and the Pharmacological Laboratory of the University of California Medical School, San Francisco.

1 Read, B. E., *Internat. J. Lep.*, 1933, **1**, 293.

2 Read, B. E., *J. Pharmacol. and Exp. Therap.*, 1924, **24**, 221.

3 Lissner, H. H., *Am. Rev. Tuberc.*, 1923, **7**, 257; Schwarz, L., *Z. nahr. Genussm.*, 1911, **22**, 441; Valenti, A., *Archiv. di Farmacol. sper. e Sci. aff.*, 1917, **24**, 23.

4 Walker, E. L., MacArthur, C. G., and Sweeney, M. A., *Trans. Nat. Tuberc. Assn.*, 1923, 553.

that tasting of injected substances does not presuppose excretion through the saliva.[5]

Other observations indicative of central action may be summarized briefly. In dogs, oral doses of 100 mg./kg. of ethyl chaulmoograte cause emesis in from 35 to 205 minutes, allowing time for sufficient absorption of chaulmoograte; vomiting does not relieve the nausea, and should the vomitus be re-eaten by the dog some time later, emesis does not then recur. Na chaulmoograte in doses of 67 mg./kg. causes emesis in dogs in an average time of 120 minutes although gastric irritation must be considerably greater than with either ethyl chaulmoograte or chaulmoogra oil. Dogs given emetic doses of chaulmoograte every third day respond by vomiting more readily; conditioning of the response was ruled out by giving an equal dose of olive oil in gelatine capsules as a control. In a dog vomiting within 35 minutes after an oral dose of 100 mg./kg. of ethyl chaulmoograte, 0.01 mg./kg. of atropine (sufficient to antagonize quantitatively one minimal emetic dose of pilocarpine) delayed emesis to 85 minutes. Two-tenths mg./kg. of atropine delayed emesis to 180 minutes, and if 0.2 mg./kg. of atropine were given with 100 mg./kg. of ethyl chaulmoograte and the same dose of atropine repeated once at 20, 40 or 80 minutes, no emesis occurs. For cats the findings were essentially the same; oral doses of 75 mg./kg. of Na chaulmoograte brought about emesis in an average time of 83 minutes although administration of 100 mg./kg. of ethyl chaulmoograte produced extreme nausea but did not result in emesis in 7 of 9 trials with 6 cats even when given on the day following administration of the same dose. Two of 9 cats so treated vomited in 65 and 87 minutes. One hundred and fifty mg./kg. of ethyl chaulmoograte produced emesis within an average time of 92 minutes in 6 of 6 cats. Intramuscular administration of the standard dose[6] of nicotine abolished the emetic response to ethyl chaulmoograte. Again, with dogs an emetic dose of morphine given one hour prior to 100 mg./kg. of ethyl chaulmoograte prevented emesis, while an emetic dose of apomorphine, which does not have a secondary depressing effect on the vomiting center as does morphine,[7] given similarly did not prevent the emetic response.

[5] Winternitz, M., Deutsch, J., and Burell, A., *Mediz. Klinik.*, 1931, **27**, 986; 1932, **28**, 831; Tarr, L., Oppenheimer, B. S., and Sager, R. V., *Am. Heart J.*, 1933, **8**, 766.

[6] Hatcher, R. A., and French, B. S., *J. Pharmacol. and Exp. Therap.*, 1932, **46**, 97.

[7] Leake, C. D., *J. Pharmacol. and Exp. Therap.*, 1922, **20**, 359.

In a human volunteer 5 mg./kg. of ethyl chaulmoograte administered orally in a gelatin capsule was tolerated with some nausea but no emesis, while 10 mg./kg. brought about emesis in 63 minutes. Dr. H. I. Cole has told us that he has observed patients tolerant to 5 cc., or about 100 mg./kg., of ethyl chaulmoograte after taking this agent orally for some time.

Among the many peculiar pharmacological actions of *Cannabis sativa* is its rôle in the *Tai-Fong-Chee* oral method of administering chaulmoogrates recommended by Travers.[8] Travers reports a mixture of 2 parts of powdered Hydnocarpus nut and 1 part of *Cannabis indica* to be well tolerated by humans. We have found 5 of 5 cats tolerate 200 mg./kg. of ethyl chaulmoograte if 100 mg./kg. of fluid extract of Cannabis is given simultaneously or previously.

Summary. Evidence is presented indicating that the emetic effect of the chaulmoogrates is central. The action of Cannabis, atropine and morphine in abolishing the emetic response in dogs and cats is reported.

7629 C

An Inexpensive Tissue for Biological Testing.

F. F. YONKMAN AND ALICE B. RICHARDS. (Introduced by A. W. Rowe.)

From the Department of Pharmacology, Boston University School of Medicine, and the Evans Memorial of the Massachusetts Memorial Hospitals, Boston.

For some time we have been interested in reactions of the iris to various reagents and have observed that a strip of sphincter pupillae of the steer iris affords an excellent preparation for studying the effects of many drugs on unstriated muscle and its innervation. We suggest the adoption of this tissue for pharmacological studies because of its inexpensiveness, certainty and sensitivity of response, availability at any abattoir and its viability even after 3 to 7 hours post mortem. Precautions regarding preparation of iris strips are given elsewhere.[1, 2]

Besides demonstrating the antagonistic relaxing action of various concentrations of atropine against sphincter contraction by physostigmine one is able to produce opposite effects with histamine and

[8] Travers, E. A. O., *Proc. Roy. Soc. Med.*, 1926, **19**, 1.

[1] Miller, G. H., *J. Pharm. and Exp. Therap.*, 1926, **28**, 219.

[2] Yonkman, F. F., *J. Pharm. and Exp. Therap.*, 1930, **40**, 195.

adrenalin. To date we have been able to demonstrate sphincter relaxation with so small a dose as 1-400,000,000 and a sphincter contracture with the minute dose of 1-1,000,000,000 of histamine. We suggest the study of the iris sphincter strip as a probable tissue for biological assay as well as its adoption in pharmacological laboratory teaching.

7630 C

Pharmacology of Inflammation: III. Influence of Hygroscopic Agents on Irritation from Cigarette Smoke.*

MICHAEL G. MULINOS AND RAYMOND L. OSBORNE.

From the Department of Pharmacology, College of Physicians and Surgeons, Columbia University.

We herewith report a successful attempt to measure objectively the irritant properties of cigarette smoke. We used the conjunctival sac of rabbits according to the technic of Hirschhorn and Mulinos.[1] In Fig. 1, smoke from the burning cigarette which is protected from drafts by a jacket *h*, is passed by way of the ammonia tube *g*, through 3 cc. of water, saline or Ringer solution, at room temperature (21 to 30°C.), and at 37.5°C. maintained by artificial means, by immersion of cylinder *f*, in a water bath. A few experiments were performed using light mineral oil as a solvent for the smoke. By means of a filter pump, tube *e* sucks air through the cigarette and through the funnel-stopcock system *b*. The tipping bucket *a* empties water into the funnel, temporarily preventing ingress of air. This sucks air first through the cigarette and then the solution. The water drops to the bottom of the cylinder and leaves through tube *d*, by gravity. When the tube *b*, becomes free from water, all the air sucked through the system goes through this tube, and none through the cigarette. This cycle yields a puff, 100 of which averaged 24.3 cc. with the limits of 21 to 28 cc., and which requires 15 seconds to complete, 4 seconds of which are taken up by suction through the cigarette. It requires 15 minutes or 60 puffs to smoke one cigarette. Through each 3 cc. of solution is drawn the smoke from 5 cigarettes in order to insure saturation.

* This research was made possible through a grant by Philip Morris & Co., Ltd., Inc.

1 Hirschhorn and Mulinos, PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 168.

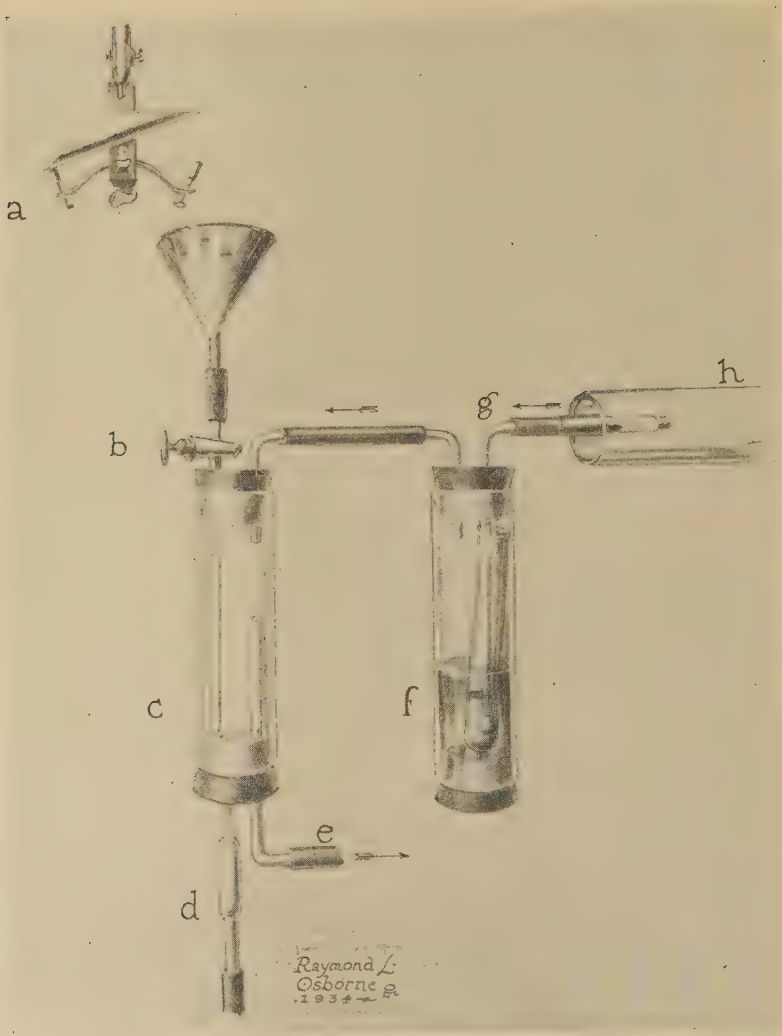

FIG. 1.

Apparatus used to puff smoke through the 3 cc. of fluid in the test tube in jacket *f*.

In order to avoid the interference of too many factors which are of necessity inherent in the problem, we have limited this first investigation to the influence of the 2 hygroscopic agents usually employed in the manufacture of cigarettes. These are glycerine and di-ethylene-glycol. These hygroscopic agents are deemed necessary to maintain the proper moisture content of the cigarette. It is possible that these chemicals may offer some interference with the combustion of the tobacco; or through their own combustion add quali-

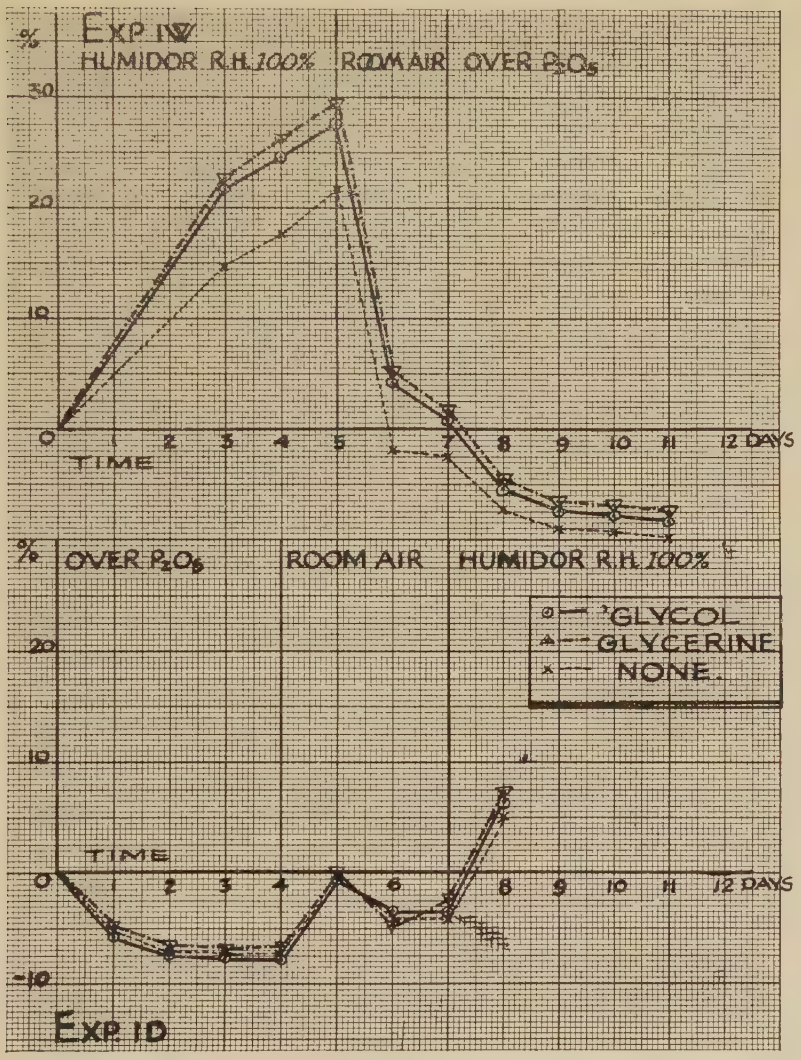

FIG. 2.

At *o*, 20 cigarettes of each variety were placed under the conditions noted on the chart.

ties to the smoke which are not present when the tobacco itself is burned.

Through the splendid cooperation of Philip Morris & Co., we obtained the necessary limitation of factors to make the investigation worth while. Under our personal supervision they prepared a batch of tobacco which was treated with the usual casing minus any hygroscopic agent. This was divided into 3 parts; to one was added

2.74% di-ethylene-glycol, to another 3.65% glycerine, and to the third nothing. From each of these, cigarettes were made in the usual manner. The above amounts of hygroscopic agents have approximately equivalent water holding powers, as is shown in Fig. 2, and are those ordinarily used in the manufacture of cigarettes. In addition we obtained some of the same tobacco to which we added subsequently 1, 3, and 5% of each hygroscopic agent.

The cigarettes were smoked under controlled conditions of humidity, and also with many variations in the water content of the cigarettes from very low (dried over phosphorus pentoxide), to very wet from standing over water for various periods of time. (Fig. 2.) However, these variations in the water content of the cigarettes had no demonstrable effect upon the irritating property of the cigarette smoke.

Two to 3 drops of the fluid through which had been sucked the smoke from 5 cigarettes were instilled into the conjunctival sac of each of 3 rabbits. The degree of blepharospasm and the amount of objection by the animal were noted. At 2 minute intervals the condition of the conjunctival mucous membrane was noted, and the edema and redness compared with that of the opposite untreated eye. In Fig. 3, the edema is reported as from plus to 4 plus.

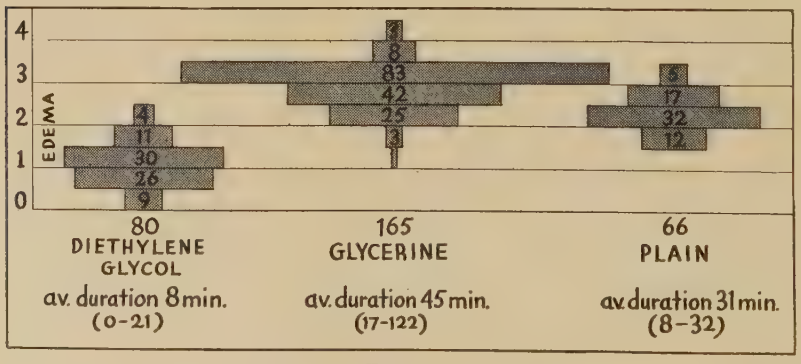

FIG. 3.
Frequency curve showing the distribution of the degree of edema in each experiment.

Results. Fig. 3 is a distribution curve of all the experiments performed. It is obvious that the cigarettes which had been made with di-ethylene-glycol as hygroscopic agent proved to be less irritating than those with no hygroscopic agent, and much less irritating than those with glycerine.

The solutions of the cigarette smoke were all acid to litmus, but no attempt was made to determine whether the acidity could account

for the inflammation produced. However, the following observation is of interest. Nicotine alkaloid in 0.005% solution which is alkaline to litmus, produced irritation about equal to that of the smoke from the di-ethylene-glycol treated cigarette. However, if the nicotine solution is made acid to litmus with an organic acid, or with carbon dioxide gas, the solution is irritating no longer, even if the concentration of nicotine is increased 10 fold. This shows that the irritating property of the cigarette smoke cannot be due to the amount of nicotine which is carried into the solution by the smoke. This conclusion is supported by the experiments of Dixon,[2] who found that the nicotine content of tobacco smoke was greater when the tobacco was moist. In our experiments, the moisture content of the cigarettes did not influence the irritating properties of the smoke, showing once more the independence between irritation and nicotine concentration in tobacco smoke.

Fig. 3 shows the maximum irritation, irrespective of time of onset or duration. The edema produced by the smoke solution from the untreated cigarette lasted an average of 31 minutes (8 to 82); that from the di-ethylene-glycol lasted 8 minutes (0 to 21); and that with the glycerine lasted 45 minutes (17 to 122).

Cigarettes made with 1, 3, and 5% glycerine respectively show a slight increase in irritation as the percent of glycerine increases. When di-ethylene-glycol is used, there is a slight but readable reduction in irritation as the percent increases. When the cigarette smoke is passed through mineral oil, the results are essentially the same as when water is used.

7631 C

Proliferation of Epithelium of Nipple of the Rat and Guinea-pig During the Oestrus Cycle.*

S. B. D. ABERLE. (Introduced by E. K. Marshall, Jr.)

From Yale University School of Medicine.

Nineteen rats and 30 guinea pigs having regular cycles were used. Daily smears were taken in the rats for 3 weeks and in the guinea

2 Dixon, *Brit. Med. J.*, October, 1927.

* This investigation was partially subsidized by a grant from the Committee for Research in Problems of Sex, Division of Medical Sciences, National Research Council.

pigs for 2 months. The animals were killed at various times during the oestrus cycle. The height of the epithelium was determined by averaging 10 cell-counts taken from sections near the center of the nipple.

In the rat the difference of the cell-count at oestrus and dioestrus is slight, but the shape of the epithelial cells changes. At dioestrum the cell is flat and the nucleus occupies most of the cell. During oestrum the cell is oblong. The change in shape increases the distance from the basement membrane to the periphery, while the nucleus appears to occupy a smaller part of the cell.

In the guinea pig the change in the shape of the superficial epithelial cells of the nipple is marked and there is an increase in number. The epithelium of the nipple of the guinea pig has rete pegs. During dioestrum the epithelium averages 10.5 cells for the height of the papilla and 4.2 cells for the intervening epithelium. The proliferation begins on the first day of the oestrus cycle. It then averages 16.1 cells for the height of the rete pegs and 7.3 cells for the height of the lower epithelium. It remains high until the fifth day after the onset of oestrum.

The epithelium from the nipples of 2 adult males, counting the lowest and highest part of the epithelium averaged 5.6 and 6.0 cells respectively. Females spayed for periods of 28, 33, and 46 days have a low nipple epithelium, averaging 9 and 3 cells in height, for rete pegs and lower epithelium respectively.

The ovaries, then, produce cyclic changes in the nipple epithelium of rats and guinea pigs; as can be seen by the change in size and number of cells during the oestrus cycle.

7632 C

Comparison of Mammary Glands of Normal and Ovariectomized Rhesus Monkeys.*

S. B. D. ABERLE. (Introduced by E. K. Marshall, Jr.)

From the Carnegie Institution of Washington.

Four monkeys were ovariectomized for periods ranging from 7 days to 6 months before autopsy. The ovaries were sectioned and found complete. The mammary glands were carefully dissected at

* This investigation was partially subsidized by a grant from the Committee for Research in Problems of Sex, Division of Medical Sciences, National Research Council.

autopsy, fixed, stained, mounted, and compared with a group of 5 unspayed controls of the same average body weight. The gland-areas from the spayed animals were not determinably different from the gland-areas of the controls. One gland from a subject spayed 6 months before death contained almost no bud tissue. Although there was a wide variation in the amount of bud-tissue present, the ducts of these ovariectomized animals did not retrogress.

TABLE I.
Comparison of Mammary Gland Area from Normal and Ovariectomized Monkeys.

	Body wt. at castration gm.	Body wt. at autopsy gm.	Period of castration (days)	Area of mammary gland sq. dm.
		Ovariectomized		
9	5050	5050	7	.83
4	5175	5320	56	.77
1	4500	5305	181	.82
11	4825	4550	198	.84
Average	4888	5056		.82
		Control		
209		3600		.65
3		4625		.43
15		5655		.98
29		5655		.95
216		4115		.40
Average		4730		.68

7633 C

Size of Mammary Glands of Normal Rhesus Monkeys and Those Injected with Theelin, Corpus Luteum Extract, and Anterior Pituitary Extract.*

S. B. D. ABERLE. (Introduced by E. K. Marshall, Jr.)

From Carnegie Institution of Washington.

Eighteen female monkeys were used. Nine, averaging 2685 gm. in weight, were injected with various hormones; and 9, averaging 2732 gm., were kept as controls. The mammary gland of the monkey grows as a flat sheet of tissue in the connective tissue beneath the skin. The area of the gland increases from puberty to maturity. It serves as an indicator of size. Group A in Fig. 1 shows the area of the mammary tissue in the controls. The size varies from 0.02

* This investigation was partially subsidized by a grant from the Committee for Research in Problems of Sex, Division of Medical Sciences, National Research Council.

to 0.24 sq. decimeter. Group B gives the area of mammary tissue from 2 animals injected for 9 days with a total of 2550 rat units of theelin. Group C shows animals injected for 9 days with 2550 rat units of theelin each and for 15 days with 1500 R.U. of corpus luteum extract each. Three animals (Group E) were injected for 44 days with a total of 9200 rat units of theelin.

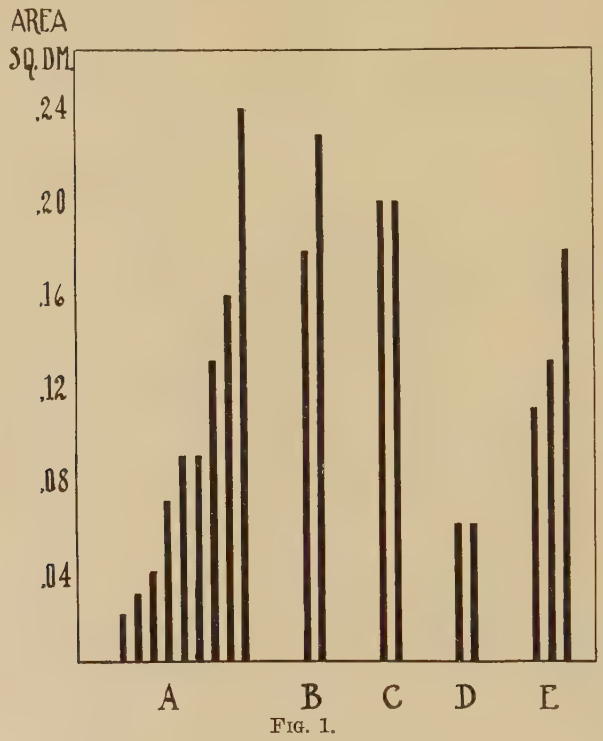

FIG. 1.

In order to discover whether follicular fluid from the monkey's own ovaries might have an effect upon the mammary gland, 2 animals (Group D) were given 4 cc. daily for 16 and 17 days of a crude alkaline extract from the anterior lobe of the pituitary gland.[1] The ovaries averaged 1.61 gm. in weight and were filled with medium sized follicles. The controls averaged 0.133 gm. in weight and showed only an occasional medium sized follicle. The area of the mammary glands was 0.06 sq. decimeter, which is within the range of the controls.

[1] The theelin and the crude alkaline extract from the anterior lobe of the pituitary gland was kindly supplied by Park Davis and Company, the corpus luteum extract by the Schering Corporation.

The buds branching from the ducts of the theelin-injected animals did not appear macroscopically to be increased in number compared with the parenchymal tissue of control animals. It is quite probable that larger doses over a longer period of time might show a marked effect.

The epithelium of the nipples in all of the injected animals was thicker than in the controls.

7634 C

Growth of Mammary Gland in the Rhesus Monkey.*

S. B. D. ABERLE. (Introduced by E. K. Marshall, Jr.)

From the Carnegie Institution of Washington.

Thirty-one females and 3 male rhesus monkeys of a wide range of body weights were used. All were kept under observation for at least 4 months. No animal dying from a protracted illness or used for other experimental purposes was included. The area of the mammary tissue was measured and compared with the weight of the kidneys and ovaries. Since the mammary tissue in the macaque grows in length and width rather than in thickness, a measure of the area of the gland gives an index of the change in size.

In 12 animals the skin surrounding the mammary tissue was tatooed, a pattern was traced, the gland with the skin was cut along the tatoo marks, dissected free of muscle, and stretched on a flat piece of cork the exact size of the pattern. The skin from the center of the tissue was removed, leaving the gland surrounded by a frame of skin. After fixation and staining the size of the gland was compared to the size of the pattern. An average shrinkage of 7% was found. Since all glands were treated in the same way this shrinkage would not affect the comparison.

After the tissue was mounted a tracing of the gland was made. The tracing was taken as if a wheel of a half-inch diameter was run about the periphery, the pencil only marking the deeper indentations. The average difference between the areas of the right and left glands from the same animal was not significant. Where only one gland had been preserved its area was multiplied by 2. In order to determine the error in the method, 2 tracings were made from 10

* This investigation was partially subsidized from a grant from the National Research Council, Committee on Problems of Sex.

glands and 2 readings with the planimeter were made from every tracing of all the glands. The average size of the first tracings taken from the tissue was 0.525 and from the second tracings was 0.532 sq. decimeters respectively. The m.v. of the individual variations was 0.015. The differences between 2 successive readings from the same tracings were 0.219 and 0.173±0.003 sq. decimeters.

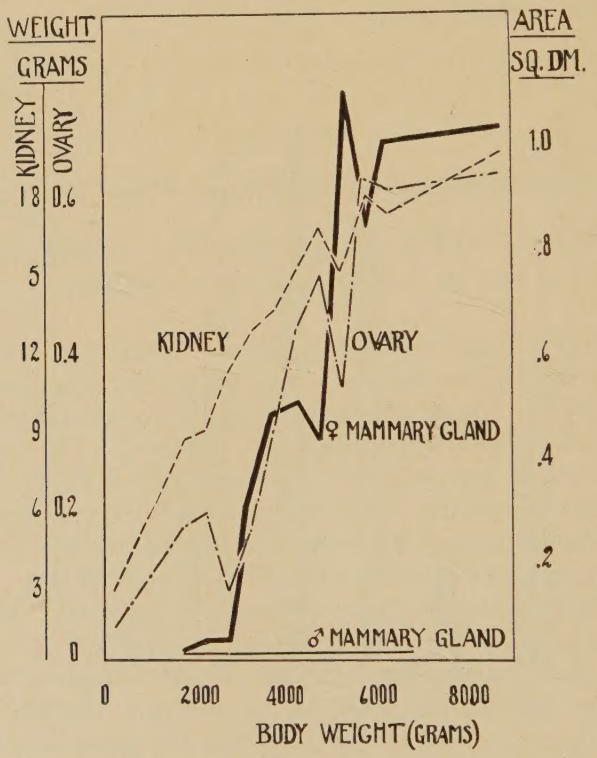

FIG. 1.

Fig. 1 shows the area of the mammary gland, together with the weight of both ovaries and both kidneys plotted against the body-weight. The kidneys show a constant rate of growth from birth; the ovaries and mammary glands show the highest rate of growth at puberty. The mammary glands are about the size of those of the male until the animal reaches 2500 gm. Then the female gland increases rapidly in size. In a group of 15 monkeys, kept in the laboratory for a year, the first menstruation occurred when the body-weight had reached 3000-4000 gm. The mammary gland continues to grow after menstruation up to a body-weight of about 5000 gm. All the animals above 4000 gm. were menstruating more or less regularly. The average area of the glands of three 2500-gm. monkeys

was 0.04 sq. decimeters. The area of the seven animals of 5000 gm. and over averaged one sq. decimeter. Thus practically the entire growth of the gland occurs while the animal is acquiring this intermediate 2500 gm.

The amount of parenchymal tissue varies widely in prepuberty as well as in mature animals. Some prepubertal glands contain chiefly ducts, with a few buds; others, although small in area, are as dense as the fullest adult glands.

The writer expresses her thanks to Dr. Carl Hartman for suggestions upon methods of investigation, for the privilege of studying his material, as well as for an open-air housing of most of the animals for long periods.